The Botanical Review

Interpreting Botanical Progress

Founded in 1935 by

H. A. GLEASON AND E. H. FULLING

Managed, edited and published at The New York Botanical Garden by

E. H. FULLING

Volume XI

1945

The Botanical Review

CONTENTS VOLUME XI, 1945

PLANT FATS IN RELATION TO ENVIRONMENT AND EVOLUTION

JAMES B. McNAIR

INTRODUCTION

In the search for proper identification of plant groups and their true sequence in evolution, the chemical and physical properties of their fats may be useful. The following paper constitutes a review of the literature directly pertaining to this thesis. In favor of the use of fats in this connection is their wide distribution throughout the plant kingdom. One of the properties of fats which are useful in such identification is their ability to absorb iodine. The iodine value or iodine number of a fat or fatty acid is the quantity of iodine (in mg.) absorbed by one gram of fat or oil under specified conditions.

However, some properties of fats, including their iodine values, are affected by environment. The literature shows that the iodine absorption values may change in relation to the amounts of fatty acids present, although the kinds of fatty acids may not be changed by environment. Under similar environments fats may be separated according to their sequence in evolutionary position. In addition to the usefulness of the iodine value as an indicator of a plant group, there is the nature of the fatty acids themselves. For instance, some fatty acids are found only in certain plant families, e.g., myristic acid in the Myristicaceae, erucic acid in the Cruciferae (38). Other properties which have been found useful in the identification of plant groups are increase in the number of carbon atoms in fatty acids and increase in the number of acids in seed fats with advance in evolutionary position (88).

The literature also shows that iodine values of fats give numerical values for plant groups, and by them more definite comparative values for evolutionary appreciation are determined than are obtained by other classification methods, e.g., serum diagnosis. For instance, the iodine numbers of their fats are for palms (Principes

or Palmales), 29; for iris (Lilliiflorae or Iridales), 81; for Rubiales, 111. These values indicate that palms are the most primitive, iris next, and the Rubiales most developed (87). The Rubiales may perhaps be considered as having developed four times as far as the palms, at least in their ability to produce unsaturated fats.

DEFINITIONS

Pure fats are solid or liquid oils consisting of a fatty (aliphatic) acid or a mixture of fatty acids chemically united to glycerine. Fats which are liquid at ordinary temperatures are also called fatty oils or fixed oils. Some fats are made up entirely or partially of unsaturated fatty acids. Unsaturated fatty acids are capable of uniting with certain other elements or compounds without elimination of any side product; thus, linoleic, linolenic and oleic acids are unsaturated. Unsaturated compounds have double or triple bonds; they are capable of uniting with iodine. Although it is possible for a fat to be made up of saturated fatty acids which do not combine with iodine, all known plant fats contain at least one unsaturated fatty acid.

FATTY OIL PRODUCTION IN RELATION TO STAGES OF DEVELOPMENT

According to the investigations of Ivanov (48), there occurs during the ripening of oil-seeds an increase of unsaturated constituents of their drying oils, whereas the composition of non-drying oils remains practically constant. If the oil of a ripe seed contains linoleic or linolenic acid, a gradual increase in the iodine number is observed in the course of development. Linseed oil in the initial stage of the ripening period gave an iodine number of 120, while the oil of the mature flax seed gave 180. Ivanov harvested flax at four stages of maturity. In 1910 the seed from the first harvest, one week after flowering, contained 4.4% of oil; the seed from the second harvest, on July 18, 13 days later, 11%; the seed from the third harvest, on August 3, 32.5%; and the seed from the fourth harvest, on August 25, 35%. These data show an extremely rapid rate of oil formation between July 18 and August 3, when practically all the oil was laid down in the seed.

Analysis of flax seed for oil content at four degrees of maturity by Eyre and Fisher (26) gave results which agree quite closely with those of Ivanov. They found that the maximum oil content

was reached and further production ceased with disappearance of the chlorophyll. The greatest rate of increase in the iodine value took place after the oil content had attained its maximum value. The value did not reach a maximum but rose throughout the whole period during which observations were made, for 53 days after flowering, although the rate of increase diminished somewhat toward the end of the period. Rapid oil formation occurred during the first 20 days when the color of the seed and oil denoted the presence of chlorophyll. After that period the seed assumed yellowish-brown and full brown and the oil a deep yellow, and the iodine value rose rapidly. The iodine value increased from 115 on the tenth day to 195 on the fifty-third day.

Bushey, Puhr and Hume (11) harvested flax planted on June 10 at five stages of maturity. The seed from the first harvest, on August 25, was very immature but contained 29.51% oil. The seed from the fourth harvest of September 21, which was fully mature, contained 36.84%. The iodine number of the extracted oil was lower at each of the successive five harvest dates. The maximum value was 181.5 at the first harvest and the minimum was 161.0 at the last harvest.

Dillman (18) has measured growth in volume and formation of oil in developing flax seed. The seed used for the study was of known age, obtained by tagging a large number of flowers and collecting the seed at frequent intervals of their development. It is evident that a study made by this method gives more accurately the rate of development of the seed, since the material used for each of the harvests is of uniform and known age. The results of Dillman's studies made in 1926 and 1927 at University Farm, St. Paul, Minnesota, and at Mandan, North Dakota, show that the growth in volume of the seed, as determined by measurements of length, width and thickness, is relatively rapid, reaching a maximum 12 to 14 days after flowering. Growth of the seed, as determined by the daily increase in dry weight, continued for 33 days and then remained constant to the end of the ripening period, 40 days after flowering. The most rapid formation of oil, based on percentage of oil in the dry seeds, began at about the seventh day after flowering and continued for 15 to 18 days. After the maximum percentage was reached, there was little or no significant change up to full maturity. The maximum percentage was reached somewhat before the maximum dry weight of the seed.

Johnson's (73) experiments with the Bison variety of flax showed the average oil content, studied at two-day intervals, to increase quite uniformly from five to 25 days after flowering, followed by a slight decrease to maturity. The iodine absorption number made at frequent periods on the seed from the three-year rotation suggests that the saturated fatty acids are formed first in the synthesis of oil, but are progressively transformed to acids with a greater degree of unsaturation. The iodine numbers increased rapidly from five to 17 days after flowering, remained fairly constant for a short period, then decreased slightly to complete maturity.

It has been shown that accumulation of oil in seeds does not set in actively during the very earliest stages in their development, and the work of Ivanov (48) suggests that there is a period of very intense oil formation which occurs about midway between blooming and final maturity of the seed. This raises the question as to the existence of a "critical period" in oil formation which would have an important bearing on the effects of external conditions on the quantity of oil produced. This question was investigated with soy beans and cotton seed (29). Samples were collected at different stages of maturity to serve more definite information on this point. The weight of the seed was also obtained in each case, so as to ascertain changes in the absolute as well as relative oil content. To arrest respiration promptly and thereby avoid changes in composition, the immature seeds were dried in an oven at 70° to 80° C. In the case of soy bean, five or six pods were picked from each of 100 to 125 plants. The material was grown at the Arlington Experiment Farm, Virginia, the Peking soy bean in 1910 and the others in 1912. The several pickings from each variety were taken from the same plants at intervals, but for obvious reasons the different pickings did not necessarily represent the actual growth made by the beans in the intervals covered. They were strictly comparable, however, as to the relation between the oil content and size of the seed at the several stages of maturity. In the case of the cotton seed, care was taken to obtain the same number of immature and mature bolls from each plant, and these were always taken from the same branch, about 12 plants being used for each pair of samples.

In all cases the relative weight of the seed rather than the date of harvesting is to be taken as the more nearly correct index of the

stages of development. The first pickings of soy bean were made when the seeds were exceedingly small, and the final pickings represented the fully matured seed. The results are definite and conclusive. Except for the period immediately after blooming and that directly preceding final maturity, there is throughout the development of the seed a gradual rather uniform gain in oil content as compared with the growth of the seed. There is no evidence of a definite "critical period" for the accumulation of oil during development of the seed. Considering only the percentage of oil, there is a very sharp increase during the first few weeks after blooming, and then only a slow gain until near the end of ripening. During the final stage of ripening there is a decrease both in the size of the seed and in the oil content. This phenomenon, which was observed also by Muntz (98), is probably due to continued respiratory activity after assimilation has ceased. In the case of cotton seed, the immature samples were taken when the green bolls had reached full size and had begun to show numerous brown spots. As in soy beans, increase in oil proceeds somewhat more rapidly than growth of the seed.

In other investigations of soy bean oil (16), two sets of ten plants each of pedigreed stock were chosen for analysis at different stages of maturity. About ten pods were picked from each plant. The first picking was made on August 23 (Wisconsin) when the seeds and pods were green; the second on September 8 when the pods were turning yellow; and the third on September 18 after the plants were fully mature and the seeds thoroughly dry.

Analyses of the seed from these two sets of plants are shown in Table IX of citation No. 16. The two sets check each other fairly well. It is significant that the iodine number increases quickly at the last stage of maturity, there being very little difference in the early stages.

One explanation which may be offered to account for the fact that the late high line has a higher iodine value than the early low line is that the quick maturity of the low line may possibly retard the complete development of the unsaturated oils, whereas the slower maturity of the high iodine line may provide better conditions for oil formation.

Besides flax seed, soy beans and cotton seed, other seeds have been analyzed in regard to their oil content in relation to the stage

of maturity. For instance, Ivanov (63), in an investigation of the oils from henbane and thornapple seed, found the maximum oil content at the middle stage of ripeness and that the acid number and iodine number decreased as ripening progressed. The iodine value of sunflower oil was found to remain almost constant during ripening (September 26 and November 15), but the saturated acids decreased steadily from 15.14% to 6.70%, linoleic from 74.74% to 64.91%, while oleic increased from 9.89% to 28.35%. After November 16 the composition of the oil remained practically uniform (6).

OIL INCREASE IN RELATION TO CARBOHYDRATE PERCENTAGE

Ivanov (48) noticed that as the percentage of oil increased in ripening flax seed the percentage of sugars decreased. This suggested conversion of sugars into oil during the oil-forming process. The change from carbohydrate to oil in seeds has been noticed by many investigators.

OIL CONTENT AS AFFECTED BY LENGTH OF GROWING PERIOD

Independently of any differences between early and late varieties as such, it might be expected that differences in oil content would be influenced by both character and length of the growing period. Several investigators have concluded that the length of the growing period is an important factor in determining the starch and protein content of wheat. In plants of determinate growth, in which all seeds are developed during approximately the same period, the effect of the length of the growing period on the oil content may be studied by making successive plantings at intervals, since many such species show a marked tendency to shorten the growing period when planted abnormally late. Mooers (97) has called attention to this tendency in soy beans, and others (29) have determined the oil content in seed of several varieties of this crop at intervals during spring and summer months.

The different plantings of soy beans showed marked variations in size of the beans and in their oil content, but there is no definite relationship between these characters and the date of planting. In other words, the character rather than the length of the season in which the seed is developed seems to be the important factor. Seed from the latest planting contain a somewhat lower percentage of oil

than others, but this relationship is not verified in other tests. These additional data all show lack of definite relationship between size and oil content of seed and length of the growing period. Garner, Allard and Foubert (29) found, besides the above, that there is a remarkable difference between varieties as to the shortening of the period required for maturing seed when planted late, *e.g.,* Buckshot and Medium Yellow varieties.

Bushey, Puhr and Hume (11), working with flax, showed, as did Garner, Allard and Foubert (29) with soy beans, that delayed planting has very little effect upon the oil content of the harvested seed. In each instance plantings were made at 15-day intervals over a two-month period beginning at the normal planting date for the crop used. Each of these studies was conducted for a single year. One variety of flax and four varieties of soy beans were included in the tests. T. E. Stoa has furnished data (unpublished) on the oil content of flax seed from several dates of planting. His studies, covering a seven-year period, show conclusively that delayed planting has only a very slight influence on the oil content of the seed.

In experiments with the effect of delayed planting on the oil content of flax seed, other analyses of seed obtained from plants sown at five successive ten-day intervals beginning on May 1 in 1929 and 1930 showed a very slight reduction in oil content with delayed planting in 1929, but no consistent differences in 1930. The iodine number of the oil determined from two varieties in 1930 indicated a slight decrease with delayed planting for Bison but no significant change for Redwing (73).

VEGETATIVE VIGOR VERSUS FAT CONTENT

Adams and Mounce (1) found a correlation between the degree of vegetative vigor and the concentration of fats and lipoids in cranberry leaves. In the cranberry, food is stored not only as starch but also as oil. In the leaves the starch seems to be transitory, and most of the stored food is in the form of oil, whereas in the stem large deposits of starch may be found. Chemical analyses of bog plants show that there is a definite correlation between the degree of vegetative vigor and the percentage of fats and lipoids in the leaf blades, the percentage of ether-soluble substances being lowest in the most vigorously vegetative plants. Leaf blades only were

analyzed from plants several years old. The amounts of ether ex-
tract on the basis of dry weight were as follows: strongly vegetative
plants 10.57%, intermediate plants 12.42%, weakly vegetative plants
14.58%.

ENVIRONMENTAL INFLUENCES ON FATTY OIL PRODUCTION

Heredity versus Environment. The growth, development and
composition of any individual plant depend on the combined action
of heredity and environment. It follows that in any investigation
of heredity and environment in relation to plant life such methods
should be followed as will make it possible to distinguish between
these two forces. The principle of clearly distinguishing between
the effects of environment and those due to heredity is simple
enough in theory, but in practice it is often extremely difficult to
follow because of interrelations between the two forces which are
not fully understood. In this way, non-inheritable variations in the
progeny of relatively pure strains grown under what would seem
to be uniform environmental conditions become especially apparent
when we come to deal with quantitative differences such as are in-
volved in a study of variable chemical composition as influenced by
the factors of nutrition. In the experiments of Garner, Allard and
Foubert on the nicotine content of tobacco, the oil content of seeds
of various species and other quantitative characters, efforts to in-
sure the greatest uniformity possible in environmental conditions
have failed to effect anything like uniform composition in the
progeny of individuals representing the purest strains available.
Some of the strains of tobacco under study had been inbred for
eight generations.

It follows that in dealing with quantitative differences in com-
position as influenced by environment, a sufficiently large number
of individuals must be used to avoid misleading results due to vari-
ations which cannot be brought under control. Where conditions
have made it impracticable to grow relatively large numbers of
plants for study the alternative of repeating the experiment can be
adopted. It is equally important to use caution in reaching gen-
eralizations based on results obtained with too small a number of
species or varieties. In the course of their work on the oil content
of seeds, Garner, Allard and Foubert frequently observed that in
soy beans (29), different varieties are not always influenced in the

same manner by environment. In such plants as soy bean or cotton, therefore, as many varieties as practicable were included in the experiments. In selecting plants for study those that were of special importance because of the oil content of their seeds and which were otherwise adapted to the work in view were taken. So far, they have made use of cotton, soy bean, peanut and sunflower. Some varieties of soy bean may be grown under a very wide range of conditions, and for this reason this plant was largely used in their experiments—grown in a number of State experiment stations.

In some cases there were differences of more than 100% in the size of the seed, and also very large differences in the percentages of oil, when soy beans were grown in different localities. It is evident that environment as well as heredity may tremendously affect the size of the seed and the quantity of oil stored therein. It should be noted, however, that the behavior of the four varieties of soy bean was by no means the same when grown in different localities. There seems to be one exception to this observation, namely, that the conditions at Pullman, Washington, were such as to produce in each case abnormally small seed.

As cotton does not thrive in a cool climate it has not been practicable to study the development of oil in the seed under such a wide range of conditions as in soy bean. It can not be stated, therefore, whether the quantity of oil stored in the seed is subject to wide fluctuations, as has been noted in soy beans. Data of Garner et al. (29) show that in a three-year test all six varieties of cotton produced considerably heavier seed, containing a decidedly lower percentage of oil, when grown in the Piedmont section of northern Georgia than when grown in the Coastal Plain region of South Carolina. The increase in size of seed in northern Georgia was not entirely offset by the decrease in the percentage of oil, so that the actual quantity of oil stored in the seed produced under these conditions was somewhat greater than in those grown in the Coastal Plain region. There was also considerable yearly fluctuation in the oil content of the seed in both sections, owing to varying seasonal conditions. As already noted, the uniformity in behavior of all the varieties of cotton contrasts sharply with the varietal differences observed in soy beans. The average difference in oil content for the six varieties of cotton as grown in the two localities is greater than the varietal difference in either locality.

Soil (Media) and Fertilizers. In dealing with the responses of plants to differences in environment, it is frequently sought to differentiate between the effects of climate and those ascribed to the soil. Climate, or the average weather, as ordinarily understood, refers to conditions of the atmosphere, of which temperature and moisture are perhaps the most important as factors of nutrition. But the soil is likewise subject to variation in temperature and moisture, and there must be a tendency toward equilibrium in temperature and moisture between these two media in which the plant lives.

It is true that under any fixed weather, or climatic conditions, plants grown on contrasted soil types may show well defined differences in their development, but such relationships are subject to change with any change in climatic factors. There is ample evidence to show that differences in plant development observed on contrasted soil types during one season may be completely reversed in another season (29). It is also true that in extreme cases difference in climate may produce certain definite differences in plant development more or less independently of the soil type. Within ordinary ranges of soil and climatic differences, however, it is hardly possible to develop far reaching generalizations as to the specific effects of either independently of the other, for change of climate results in a change of soil conditions, and *vice versa.*

In spite of the above mentioned limitations which must apply in considering soil and climate as environmental factors, it seemed desirable to Garner *et al.* (29) to obtain data as to the influence of differences in soil type on the accumulation of oil in the seed as produced under varying seasonal conditions. In the experiments with cotton six representative Upland varieties were grown for three consecutive years (1909–1911) at Thompson Mills, Georgia, on two adjoining but contrasted types of soil, the original lots of seed being used for each year's planting. For convenience these soil types were designated as "red soil" and "gray soil," respectively. Both belong to the Cecil series and were in a good state of cultivation. The red soil is comparatively heavy, tenacious clay, while the gray soil is an open-textured sandy loam. As all the varieties were affected in a similar manner, the results are averages for the six.

Taking the average results for the three years, the red soil gave

only slightly heavier seed, with a somewhat smaller proportion of hulls than the gray soil, and there was practically no difference in the oil content. Each year the seed was heavier and contained a smaller proportion of hulls on the red soil than on the light soil, but the case is quite different as regards the oil content. In 1909 the oil content was considererably higher on the red soil than on the gray, while in 1910 these relations were reversed and in 1911 the differences practically disappeared. In other words, the comparative effects of the two soil types depend on the seasonal conditions, and it so happened that there was a balancing effect for the three years covered by the experiment.

The tests with soy beans included a wide range of soil types and climatic conditions, and the results as a whole emphasize the fact that the relative effects of different soil types are not specific and constant but depend largely on seasonal conditions, as was brought out in the experiment with cotton. The results in the field experiment at Arlington Farm, as compared with those obtained in the greenhouse with the same soils, illustrate this point. In the field tests the plants suffered considerably from drought during the growing season, and the sandy soil gave decidedly smaller beans and higher relative oil content than the clay soil. In the greenhouse the difference in size of beans largely disappeared, while the clay loam gave a somewhat higher percentage of oil than the sandy soil. In the pot experiments at Arlington Farm the lighter soils gave somewhat larger seed with lower percentage of oil than the heavier clay soil, but at Manning, South Carolina, there were no significant differences.

Experiments similar to those with soy beans were made with the Spanish variety of peanut. The effects produced by the different soil types are of the same general character as with soy beans, although the behavior of the two species under similar conditions is not always the same. In 1913 a series of pot cultures with sunflower were carried out at the Arlington Farm (29) in the same manner as for soy beans, using a number of different soils in the test. Soil differences brought about very marked differences in the size of the seed, but the variations in relative oil content were less decided.

On comparing the data of oil content of soy beans grown under different environmental conditions with related data obtained

with different soil types it becomes apparent that variations in the size of seed and the oil content of soy beans attributable to differences in soil type are far less than those observed when both soil and climate differ. The same relationships are observed in cotton seed. These results are interpreted as indicating that under practical conditions climate is a more potent factor than the soil in modifying the size of seed and its oil content. The most probable explanation is that the atmosphere is subject to greater and more rapid variations in moisture and particularly in temperature, and also that the "soil climate" is greatly influenced by the weather conditions. Temperature and moisture differences of both soil and atmosphere are among the important factors of environment which may influence the plant characters under study, and this factor complex must be at least partially analyzed before satisfactory conclusions can be reached as to the principal external factors concerned in oil formation in the plant.

Bacteria. A colorless strain of *Serratia marcescens* was grown on agar and the lipoid content determined (31). On meat extract at pH 7 the lipoid content reached 0.6% (dry basis) in eight days. With increasing age the lipoid fell. Age and pH are so related that the lipoid content remains nearly constant near neutrality but decreases at acid or alkaline reactions. On fluid media of the same composition two to four times as much lipoid is formed.

Mold. Single cell cultures of *Aspergillus fischeri,* grown on synthetic media containing glucose as the sole source of carbon, showed differences in the amount and nature of the fat, depending on the initial concentration of glucose, concentration of ammonium nitrate, pH, temperature, increased aeration and period of incubation (104). A high fat content was favored by neutral or slightly alkaline media, a high concentration of glucose and a low concentration of ammonium nitrate. The percentage of sterol in the mycelium was increased by the use of a fairly high initial concentration of glucose, 1.0% of ammonium nitrate or an initially acid medium containing urea, a higher temperature (37° C.) and a long period of incubation. The percentage of lipinphosphorus in the mycelium was increased by the use of a low initial concentration of glucose, 1.0% of ammonium nitrate and an initially slightly acid medium.

Fertilizers. The experiments in different soil types with soy beans, cotton, *etc.*, previously described, have included soils varying greatly in fertility, as indicated by the comparative growth of the plants, and the results as a whole show that within the limits ordinarily met in farm practice the relative fertility of the soil does not greatly influence the size of the seeds or oil content (29). A large number of fertilizer tests with cotton were carried out at Lamar and Timmonsville, South Carolina, in 1909, 1910 and 1911, to obtain more accurate information as to the effects of fertilizers on the size and oil content of seed. The results are averages of duplicate plots, except for the controls, which represent the averages for four plots in each case, all plots being 1/40 acre. The tests in 1909 and 1910 included plots receiving four different quantities of nitrogen, four of phosphoric acid, and three of potassium. Dried blood, acid phosphate and muriate of potash were used as fertilizers.

The soil used in 1911 was very poor, as shown by the large increases in crop yields produced by the complete fertilizers. Addition of all three elements, nitrogen, phosphorus and potassium combined in various proportions, gave in all cases considerably heavier seeds, with a smaller percentage of hulls and a higher oil content in the kernels as compared with the controls. With respect to the varying quantities of the three fertilizer elements, increased applications of nitrogen had no appreciable effect on the weight of the seed and only a slight effect on the percentage of hulls, but lowered considerably the oil content of the kernels. Increased applications of phosphorus and potassium did not materially affect any of these characters. The tests of the two preceding years gave similar results.

Pot culture tests were made in 1911 with the Peking variety of soy beans, using tile cylinders filled with Arlington clay soil. In a series in which phosphorus and potassium in a fixed ratio were added in three different quantities, the yields of beans were greatly increased and the weight of the seed was not changed, while the oil content was increased about 20%. With addition of phosphorus alone, very much the same results were obtained; but with addition of potassium alone there was only a small increase in yield and practically no increase in oil content. In similar tests with Spanish peanuts phosphorus gave a large increase in yield

and slightly increased the weight of the peas, but had no effect on the oil content. Potassium had practically no effect on the yield, the weight of seed, or the oil content.

In his study of fat production in *Picea excelsa* and *P. sitchensis* seedlings grown in cultures, Laing (77) found in both species under all treatments that fats were in the leaf, except where potassium was omitted from the culture. But there was a stronger reaction for fats in the root system of plants in potassium-free cultures than in the presence of potassium. Fats were absent from the root tissues of *P. excelsa* when phosphorus was omitted from the culture.

Linseed oil was found to have a somewhat higher iodine number when produced on non-fertilized fields than when produced on fertilized fields. They were, respectively, 189.7 and 188.1 (7).

Temperature. The temperature at which fats are formed is one of the factors which determine their degree of unsaturation. This is based upon three separate lines of reasoning (79). First, a general survey of the fats in animals and plants indicates that the highly unsaturated fats are most commonly found in plants growing in temperate or cold regions (103, 52, 83) and in the poikilothermic animals which live under relatively cold conditions, while the most saturated fats are found in tropical or subtropical plants and in warm blooded animals. The carnivora should not be included in this generalization because the composition of their adipose tissue fat is partly, at any rate, determined by the fat which they take in as food, and even in animals, such as the pig, which produce fat easily from carbohydrate, differences have been observed in the composition of their stored fat which may be referred to differences in the composition of the fats present in the cereals on which they have been fed. Secondly, it is usual to find that the fat in living organisms is fluid at the temperature at which the organisms exist, except endosperm fats. This would preclude the formation of saturated acids to any great extent in organisms living under temperate or cold conditions, because such acids would tend to raise the melting points even of mixed glycerides which contained them. The higher melting points of the saturated acids would be less important from this point of view in the warm blooded animals or tropical plants, and it might be more likely, therefore, to expect saturated acids to occur to a

greater extent in organisms living under warmer conditions.
Thirdly, various hypotheses have been made regarding the chem-
ical reactions by which fatty acids are formed in nature, namely,
autocondensation of acetaldehyde (78), condensation of pyruvic
acid and acetaldehyde (111) or autocondensation of pyruvic acid
(99). All these admit of the possibility of formation of long
chains of carbon atoms with unsaturated linkages as products of
the condensation reactions which produce them, and if they are
to give rise to saturated carbon atom chains a reduction must take
place subsequently. If this represents what takes place, then the
unsaturated acids would be precursors of the saturated. Two
different types of reaction are involved, namely, condensation and
reduction. It seems possible that both these reactions would not
necessarily take place with the same ease at low temperatures, and,
if it be assumed that the condensation takes place readily at low
temperatures whereas reduction requires higher temperature, an
explanation of the distribution in nature of the saturated and un-
saturated acids referred to above might be found on these lines.

   In experiments with fungi it has been noted that the more un-
saturated fats are produced at a lower temperature. By com-
paring the energy loss from the medium during growth with the
potential energy of the fungus formed at different temperatures,
the expenditure of energy was somewhat greater at the higher
temperatures (116). This indicated that production of the more
saturated acids under these conditions involves greater utilization
of the potential energy of the medium and suggests that reduction
succeeds condensation in the series of reactions by which fatty
acids are produced, and thereby involves greater expenditure of
energy. However, according to others (63), lower saturated
fatty acids are first formed and the higher unsaturated acids later.
This view is supported by still others (107, 25, 6).

   Mild warm climate favors the formation of saturated fatty acids
and of unsaturated acids with one double bond. Cold climate
favors formation of unsaturated acids with two or three double
bonds (56).

   Linolenic acid, $C_{18}H_{30}O_2$, is the most sensitive to climatic con-
ditions (52), the reason being that this acid with its three double
linkages is easily oxidized and readily absorbed by plants where
it develops heat. The action appears whenever plants are obliged

to accommodate themselves to cold climates, and may date back to the conifers at the end of the Paleozoic age. Linolenic acid is said to be the first vegetable product met with which permits an analysis of "accommodation" from the chemical point of view.

Oils containing glycerides of unsaturated acids with one double bond (oleic, erucic or ricinic) were indifferent toward climatic changes (57), while with acids having three double bonds (α- and β-linolenic acids) the iodine number decreased with increase in temperature or with the growth of the plant in more southerly regions. Oils with two double bonds (of the linoleic acid type) held an intermediate position.

Pigulevski (102, 103) found that a plant growing in a cold climate produces fatty acids of a lower degree of saturation and therefore of greater chemical activity than the same plant growing in a warm climate.

Ladd (76) arrived at similar results in experiments with soy beans. Soy plants from warm regions that gave an oil having a rather low iodine number were later transferred to colder localities. They then produced seeds whose oils gave higher iodine values. Conversely, a lowering of iodine numbers was obtained from oils from such seeds which had been shifted from north to south.

Low temperature (20° C.) was found to increase the iodine values of the fatty acids produced by the mold *Aspergillus fischeri* (104). The iodine value was 93 at 20° C. and 88 at 37° C. The iodine values were likewise increased when the mold was grown on a low concentration of glucose (1.0 to 70.0 gms. per 100 ml. of solution) or on a medium which became strongly acid (*e.g.*, NH_4Cl as nitrogen source). The neutral equivalents of the fatty acids were in all cases very near to 280, suggesting a preponderance of fatty acids which contain 18 carbon atoms.

Tuttle (118) examined a number of trees, shrubs and perennial herbaceous plants of the region near Edmonton, Canada. All species examined showed a high starch content during summer, which disappeared in October. With the exception of *Lonicera glaucescens* and *Crataegus* sp. all the trees and shrubs contained oils and fats as food reserve during winter. The other genera examined were *Syringa, Populus, Prunus, Salix, Shepherdia, Ribes, Picea, Pinus, Rosa, Pyrola, Cornus* and *Eleagnus*. In his

study of the evergreen leaves of *Linnaea* (117) he found starch formed at moderately elevated temperatures and that such starch is converted into oil when the temperature is gradually lowered. The oil is reconverted into starch when the temperature is again raised.

In a study of the occurrence of starch and oil in seeds it was noted that starch was present less frequently in the seeds of temperate plant families than in those of tropical plant families (85).

We have in the so-called bioclimatic law of latitude, longitude and altitude an example of a natural law which represents the general laws of climate as affecting the seasonal activities of plants. In the United States this variation, other things being equal, is at the rate of four days for each degree of latitude, 5° of longitude and 400 ft. of altitude (41, 42). We can comprehend the effect of this law on both qualitative and quantitative fat formation.

Latitude. As plants grow nearer to the equator the calorific value and the iodine number of their fats decrease (50, 52, 53, 55, 64). This is shown in Table I.

When plants are transplanted from a northern climate to a southern or *vice versa,* the oil content changes to correspond with the climate (76, 55, 52, 53).

Oil from the nuts of *Pinus cembra* of western Siberia was found to consist of oleic, linoleic and linolenic glycerides. Of these the content of linolenic acid was highest in the most northern climate (66).

The seed fats of seven species of *Paeonia* showed increasing iodine values in going from south to north and from sea level to higher altitudes (69, Table I). These plants grow wild in the Siberian forests, in Altai, Ural, Caucasus and in the Crimea. They produce a great many seeds which contain from 26% to 41% of fat.

In coconut palm oil the unsaturated fatty acids increased from south to north and the iodine value likewise (62). The iodine value at the equator is 4 to 10 for *Cocos bonetti,* in Suchum it is 23.6.

In a study of the fatty oils of the Labiatae and Anacardiaceae (64), the content of fatty acids with three double bonds was found to diminish as one goes from the northern countries toward the tropics.

TABLE I

INFLUENCE OF LATITUDE AND ALTITUDE ON THE IODINE VALUE OF PLANT OILS

Species and family	Place of cultivation	North latitude (degrees)	Altitude (meters)	Fat (%)	Iodine value
Iris ruthenica (Iridaceae)	Shebalino (Altai)	50–52°	800	....	106.2(33)
Paeonia anomala (Ranunculaceae)	Katum River	51°	1200	....	108.1(33)
	Seminiski Pass	....	1860	....	117–119(33)
	Seminiski Pass (Altai)	50–52°	800	31.7	142.2(69)
Paeonia anomala var. Beresovsky	Gov. Perina	....	1870	41.1	144.4–145.8(69)
	Seminiski Pass (Altai)	50–52°		32.4	157(69)
Paeonia tenuifolia	Leningrad	60°		24.5	153.5–155.2(69)
Paeonia corallina var. triternata	Crimea, Karadag	....		34.0	145.8(69)
" "	Moscow	55°50′		29.7	148.8(69)
" "	Karadag	....		28.7	127.7–128.9(69)
Trollius astaticus (Ranunculaceae)	Sympheropol	60°		26.3	130.3(69)
	Leningrad	54°	about 50	24.6	138.7(69)
	Simbirsk	....		....	134.5(58)
Glaucium luteum (Papaveraceae)	Seminiski Pass	51°	1860	....	149(58)
	Tiflis	41°40′		....	134–138.5(58)
Roemeria rhoeadiflora (Papaveraceae)	Moscow	50°50′		....	145.9(58)
	Turkestan	41°		....	144.4(58)
Thlaspi arvense (Cruciferae)	Moscow	55°50′		....	157.7(58)
	Erfurt, Germany	51°		....	103.6(67)
	Rostov (Donu)	47°10′	about 50	....	110.5(67)
	Bakuriani	41°25′	1670	....	128.9(67)
	Ust-Tsylma	65°10′	....	....	134.2(67)

TABLE I—(*Continued*)

Species and family	Place of cultivation	North latitude (degrees)	Altitude (meters)	Fat (%)	Iodine value
Erica sativa (Cruciferae)	Tashkent	41°	…	…	96–97 (33)
Brassica campestris (Cruciferae)	Samarkand	40°	…	…	97.4–98.1 (58)
	Moscow	55°50′	…	…	98–99 (33)
	Moscow	55°50′	…	…	100–101 (58)
	Mindau, Turkestan	…	…	…	95–102.5 (67)
	Cuba	45°	300	33.0	94 (58)
	Moscow	55°50′	…	…	96 (58)
	Transbalkans	84°	…	…	98 (58)
	Biisk	52°	…	…	100.4 (67)
	Moscow	52°	…	…	101.0 (67)
	Transbalkans	56°	…	…	104.3 (33)
	Moscow		…	…	104.4 (33)
	Buitenzorg	55°50′ 7° So. Lat.	…	…	89.1 (67)
Brassica rugosa (Cruciferae)	Rostov (Don)	47°	…	…	98.1 (67)
	Prain in Biisk (Altai)	52°	…	…	100.4 (67)
	Buitenzorg, Java	7° So. Lat.	…	…	98.1 (67)
Raphanus sativus (Cruciferae)	Biisk	52°	…	…	114 (67)
	Poltava	49°30′	…	…	128.14 (67)
Capsella bursa pastoris (Cruciferae)	Novgorod	59°	…	…	138–139.3 (67)
	Karadag (Crimea)	45°	…	…	133.12 (67)
Camelina sativa (Cruciferae)	Poltava	49°30′	…	…	140 (33)
	Tambov	53°	…	…	149.8 (67)
	Moscow	55°50′	…	…	154.1 (33)

TABLE I—(Continued)

Species and family	Place of cultivation	North latitude (degrees)	Altitude (meters)	Fat (%)	Iodine value
Neslea paniculata (Cruciferae)	Rostov (Don)	47°10'	about 50	....	130 (58)
	Bakuriani	41°25'	1670	....	133.5 (67)
	Bakuriani	41°25'	1670	....	139.5 (58)
	Moscow	55°50'	about 190	....	141 (67)
	Bakuriani	41°25'	1670	....	148.2 (67)
Hesperis matronalis (Cruciferae)	Moscow	56°		....	149–155.3 (67)
	Tashkent (Central Asia)	41°		....	154–158 (58)
Linum usitatissimum (Linaceae)	Tiflis	41°40'	sea level	....	154–160 (33)
	Tiflis	41°25'		....	154–164 (33)
	Kursk Tula	36°–37°		....	174–176 (58)
	Bakuriani	41°45'	1670	....	179.4 (58)
	Moscow	55°59'		....	176–184 (58)
	Bisk-Barnaul	85°		....	185–194 (58)
	Archangel	65°		....	195–200 (58)
	Java	7° So. Lat.		....	85–86 (58)
Ricinus communis (Euphorbiaceae)	Persia	35°		....	84–87 (58)
	Turkestan	41°		....	86 (33)
	North Caucasus	45°		....	88 (33)
	Moscow	55°50'		....	87–88 (58)
	Tashkent	41°		....	116.1 (33)
Althaea ficiflora (Malvaceae)	Moscow	55°50'		....	119.4 (33)

TABLE I—(Continued)

Species and family	Place of cultivation	North latitude (degrees)	Altitude (meters)	Fat (%)	Iodine value
Olea europea (Oleaceae)	North Africa	29°	...	...	84–89 (58)
Luffa acutangula (Cucurbitaceae)	Suchum	43°	...	...	85 (58)
	Italy	43°	...	...	84–89 (58)
	Tashkent	41°	...	...	120.3–122 (33)
Ecballium Elaterium (Cucurbitaceae)	Buitenzorg	7° So. Lat.	...	...	133.3 (33)
	Palermo	38°	...	...	138.2 (33)
Cucumis sativa (Cucurbitaceae)	Tashkent	41°	...	...	144.9 (33)
	Buitenzorg, Java	7° So. Lat.	...	...	115.1 (33)
Cucumis citrullus (Cucurbitaceae)	Moscow	55°50′	...	...	132 (33)
	Saratov	51°40′	...	...	122.4 (33)
Carthamus tinctorius (Compositae)	Biisk (Altai)	52°	...	24.7	127.7 (33)
	Central Africa	.....	...	...	115.1 (68)
Helianthus annuus (Compositae)	Tashkent	41°	...	30–32	124.2 (68)
	Ashabad	38°	...	...	118 (58)
Madia sativa (Compositae)	Voronezh	51°40′	...	...	126–130 (58)
	Omsk	55°	...	...	140.4 (58)
	Central Africa	.....	...	...	81.5 (68)
	Poltava	49°30′	...	...	117–134 (68)

In a study of the fatty oils of the Cruciferae and Compositae (67, 68), the iodine values of the oils of both families increased from southern to northern locations and from lower to higher altitudes. The least variation was in oils which contained fatty acids with one double bond, and the greatest variation in acids with triple bonds.

The percentage of oil in different varieties of flax seed was found by N. N. Ivanov (45) to remain nearly constant in various climates (arctic, subtropical, maritime and continental). He considers the oil content in these plants to be an inherited property. However, the iodine value of the oil was found to change. It increased when plantings were shifted to the north. S. S. Ivanov concluded from his work on flax seed in 1932 that this seed, when produced at high temperatures, formed negligible amounts of linolenic acid. On the other hand, in northern or alpine climates large amounts of unsaturated fatty acids are formed. This corroborates the general rule that warm climates favor formation of oleic acid, while northern climates favor that of linolenic acid. Linseed from Nolinsk (Russia) was cultivated at two stations in Switzerland, at Liebefeld, 550 meters altitude, and at Davos, 1550 meters altitude; also in the tropical house of the botanical gardens at Berlin. The iodine numbers (Hübl) of these oils were: Nolinsk 185.1, Liebefeld 188.4, Davos 189.6, Berlin tropical house 92.57. The weather at the Swiss stations was rainy and cold, whereas the temperature at the Berlin house was kept at 25°–30° with an atmosphere saturated with water vapor.

Fachini and Dorta (27) found that olive oils from the northern parts of Italy (Lake Garda, Liguria) contain only small proportions (2% to 6%) of linoleic acid, whereas certain oils from southern Italy, Greece and northern Africa contain considerable quantities, even as much as 17%, together with a high percentage of saturated fatty acids. These authors maintain that the composition of olive oils seems to be influenced by the age and degree of acclimatization of the plant. The increase in the percentage of linoleic acid with approach to the equator is the opposite observation to that usually found. However, the increase in the amounts of saturated acids near the equator is in harmony with the general rule, and perhaps these so dilute the unsaturated acids present that the iodine value for the southern oil is less than that of the northern.

Altitude. Analyses of petroleum-ether extracts of the wood of *Cedrus deodora* growing in the Himalayas gave an increase in the iodine value (Hübl) from sapwood to heartwood and with increase in altitude. All samples were taken in January and gave the following results: 5000 ft. sapwood 101.3, heartwood 101.9; 7000 ft. sapwood 101.4, heartwood 102.2; 9000 ft. sapwood 101.6, heartwood 102.9; 10,000 ft. sapwood 109.7, heartwood 113.0 (*92, 93*).

Increase in altitude has been found to increase the iodine number of seed fats in many plants (Table I). Altitude increase, latitude increase and temperature decrease have all been found to increase the iodine number, and *vice versa.*

Submergence. Haas and Hill (34), in their study of some British brown algae selected with regard to their vertical distribution, showed an increase in fat and fat-like substances with the degree of emergence accompanied by an increase in the saturation of these substances and an increase in unsaponifiable residue with the depth of immersion.

The Phaeophyceae of Britain are zoned from the sublittoral through the littoral to the salt-marsh region, and the conditions of life vary accordingly. At the lowest limit, the laminarias are emersed only at the neap spring tides; consequently, they are exposed to the air for only short periods, and run but small risk of desiccation; they are subject to a narrow range of temperature and to a wide range in light intensity. Thus, for the most part, they live in serene circumstances, almost a thalassic Avilion.

At the highest limit, fucoids of the salt marsh are submerged only at high tides, maybe only by the spring tides, *Pelvetia canaliculata* f. *libera,* for example. During their long exposure they may experience wide ranges in temperature, and the coincidences of weather conditions may produce such periods of drought that the plants may become almost brittle as a result of desiccation. Their existence is subaerial rather than aquatic.

The conditions of life in the littoral zone vary between these extremes, and need no further comment.

The Rhodophyceae, although for the most part sublittoral, also show some zonation: *Polysiphonia fastigata, e.g.,* often is epiphytic on *Ascophyllum* and *Bostrychia scorpioides,* a salt-marsh plant, often growing in association with *Pelvetia canaliculata* f. *libera.*

Contemplation of these extremes lead to many questions, especially concerning the correlation between metabolism and habitat.

The following table shows various fucoids arranged, as nearly as may be, in order of zonation downwards. For the sake of comparison, two members of the Rhodophyceae, which normally occur at the extremes of zonation, are added, together with two of the Chlorophyceae which were collected at the highest tide levels. The ether extract consists of fat and fat-soluble substances, including pigments. The results for the most part are for gatherings made at the same season, although not necessarily, for obvious reasons, from the same locality:

PLANT	ETHER EXTRACT
	%
Pelvetia canaliculata f. *libera*	8.62
Pelvetia canaliculata	4.88
Fucus vesiculosus f. *volubilis*	3.76
Ascophyllum nodosum	2.87
Fucus vesiculosus	2.60
Halydris siliquosa	2.18
Himanthalia lorea	1.21
Desmaresta aculeata	0.65
Laminaria digitata	0.46
Bostrychia scorpioides	0.31
Chondrus crispus	0.204
Enteromorpha intestinalis	0.217
Ulva latissima	0.185

The amount of ether extract detected in *Laminaria* corresponds quite closely to that obtained by other investigators. For instance, König and Bettels (75) found 0.39% to 0.5% in air-dried material, and Hoagland (40), in his work on the kelps of the Pacific coast, gives the following percentages in terms of dry weight:

PLANT	ETHER EXTRACT
	%
Laminaria Andersonii	0.65
Macrocystis pyrifera	0.34–0.40
Nereocystis Luetkeana	1.06
Pelagophycus porra	0.27

Some seasonal variation occurs, however, as is shown by the following:

Pelvetia canaliculata	October	4.88
	May	5.84
Laminaria digitata	March	0.46
	July	1.36

Such variation, however, was not noted in *P. canaliculata* f. *libera*, the average value for October being 8.62% and for May 8.65%. This is hardly surprising, for the dominant factor in its life is periodic emersion, generally for relatively long periods.

QUANTITATIVE FAT AND IODINE VALUE

	1	2	3	4	5	6
	Ether extract	True fat	Un- sapon. of 1	Fatty acid of 1	Iodine value of 1	Iodine value of 4
Pelvetia canaliculata f. *libera* ..	8.0	6.2	7.6	72.5	106	107
Pelvetia canaliculata	4.9	3.6	10.8	69.9	115	124
Fucus vesiculosus	2.6	1.9	16.9	71.6	114	108
Laminaria digitata		0.16	25.9	49.9	123	110

These figures relating to the Phaeophyceae are of considerable interest in that they show a direct correlation between the amount of fat and vertical distribution of the weeds, *i.e.*, duration of exposure. The greater the duration of emersion the greater is the exposure to conditions favoring desiccation and to a wider range of temperature. Here there is a remarkable parallel with what may occur in land plants, for the chemical nature of the reserve food in many evergreen plants varies with climatic conditions, and fat and fat-like substances may appear in the leaves on the advent of winter, often at the expense of starch (117, 118, 94, 20).

The iodine value of the ether extract of the Phaeophyceae falls with the degree of emersion, being highest in *Laminaria* and lowest in *P. canaliculata* f. *libera*, which means that the fat and fat-like substances of the latter plant are the more highly saturated. This may be correlated with the more extreme conditions of its life, especially a higher temperature for the most part of the year and prolonged periods of desiccation.

It is to be pointed out, however, that the iodine values of *P. canaliculata* and *F. vesiculosus* are practically identical, but the measure of their vertical separation is not more than a few feet.

The relation between the degree of saturation and vertical distribution would not appear to obtain when the iodine values of the fatty acids of the selected plants are compared. It is uncertain what significance can be attached to these figures, for during isolation of the fatty acids from the original ether extract they became more saturated, although reasonable precautions were taken.

For the rest, it may be mentioned that there is a greater propor-

tion of liquid fatty acids in *P. canaliculata* f. *libera* than in *L. digitata*.

These observations apply only to the Phaeophyceae. The two forms of Rhodophyceae examined, *Bostrychia* and *Chondrus*, representing the extremes of the habitat, contain but little fat, and the disparity between the amounts is not so great as in representatives of the Phaeophyceae. Similarly, the two great algae, *Entermorpha* and *Ulva*, characteristic of the upper tidal reaches, contain but little fat, and there is no corresponding plant of the lower tidal limits with which to institute a comparison.

Shade. Slight shading was found to increase the starch content of flax seeds, while the oil content was lowered. The quality of the oil was not influenced, but with heavy shade the iodine number was below normal (50).

Moisture. According to Antevs (3), the temperature and water content of trees are the most important factors in determining the ratio between reserve substances. He found that starch solution and fat formation during winter are more pronounced in the same species in Stockholm than in middle Europe. Fat solution and starch regeneration in spring depend to a great extent on climate. "Fat trees", *e.g.*, *Alnus*, can exist during winter without the type of fat which gives a reaction with Sudan III, and also without starch. Instead they possess an unknown fatty substance, colored yellow by Sudan III. Some "fat trees" (*Salix caprea* and *Prunus padus*) contain this unknown fat in moderately large quantities, besides typical fat and starch. Contemporary with starch regeneration the unknown fat is transformed into typical fat and starch.

Sinnott (108) suggests that the type of food reserve may be due to differences in the water content of the storage cells giving a modification of enzyme action or to differences in the ease with which enzymes have effective access to the storage cells. Where the movement of liquids is slow, starch predominates, and where it is easy, starch disappears at the beginning of winter and fat is produced. From a study of the distribution of starch and fat in woody plants, Sinnott concludes that during winter starch is commonest in regions remote from centers of production, and in cells with thick lignified, small pitted walls, and that fat is most abundant in and near the phloem, close to the vessels, and in cells with thin or unlignified walls or large pits.

Although the oil content of the same plant generally decreases from northerly to southerly latitudes, increasing moisture content of the soil has been found to increase with the total oil in the seeds (47). In flax the iodine number of the oil increases with the moisture content of the soil. In the irrigated southern districts of Russia the oil content of flax reaches 40% and the iodine number is 181.

In the 1933 crop of Canadian flax seed Geddes (30) found the iodine number of the oil somewhat lower than those of previous crops from the same localities. This, he thinks, may have been caused by a deficiency of soil moisture, which would tend to increase the ratio of oleic to linoleic and linolenic acids and deleteriously affect the drying quality of the oil.

The percentage of oil in oil-bearing plants grown under irrigation was found to increase up to a certain number of irrigations, beyond which it decreased (46). The iodine number did not increase to an appreciable extent with increase in the number of irrigations except in flax.

A study of the maturing seed of *Linnaea* and hemp showed the quantity of oil to increase to a certain maximum as the seed became dry (19). In immature seed, dried after shelling, the amount of oil is not increased because normal maturation is arrested. Under ordinary conditions of germination the amount of oil is diminished to one-half after two hours of swelling and is negligible after 24 hours. If the presence of oxygen hinders germination, the quantity of oil remains the same, although the grains have absorbed enough water. In normal conditions of germination water is indispensable for transformation of oil. In winter the amount of oil in birch and linden trees is increased and at the same time water is diminished. In spring, with absorption of water, the amount of oil is decreased.

As in many higher plants, so in yeast, the equilibrium carbohydrate ⇌ fat is controlled by variations in water content. When cultivated under conditions which kept the water below 85%, yeast showed an enormous increase in fat and sterol content (35). The total lipoid can thus be increased 20 times and the sterol 60 times the original values.

On solid media, *e.g.*, nutrient agar, the sterols increase more rapidly than the glycerides, but acidity rapidly increases also.

The low water content required for the fat and sterol enrichment is obtained by pressing the yeast and spreading it on porous plates.

Spacing. Dense sowing of flax was found to increase the iodine number of the oil to an average of 189.9, while thin sowing lowered the average to 187.9 (7). Fomin (28) noticed that variation in the spacing of sunflower plants had considerable influence on the oil content of their seeds.

HEREDITARY VARIETAL DIFFERENCES IN FATTY OIL CONTENT

Except for experiments on plant pigments and considerable practical breeding for sugar in beets, there has been little direct research on the inheritance of chemical characters in plants.

Corn—oil percentage. The Illinois corn breeding experiments (112) on selection for high and low protein and high and low oil content stand preeminent in this field. Starting with a variety of average composition, it was possible by selection and breeding in ten generations to increase the average oil content from 4.70% to 7.37% and to decrease it from 4.70% to 2.66%. These results were conclusively shown to be quite independent of soil, climate or cultural conditions.

By analyzing the pedigree records of the Illinois experiment, Surface (115) was able to show that the selection process had merely isolated already existing types of oil and protein percentage, the intermediate types having been discarded during the years of selection. This process is characteristic of an open-pollinated crop like corn.

Pearl and Bartlett (100) describe a preliminary undertaking on the heredity of chemical characters in maize. From their experiments they infer that the seed characters with respect to moisture, nitrogen, protein, crude fat, ash, crude fiber, pentosans, sucrose, dextrose and starch are inherited in maize essentially in accordance with Mendelian principles. They state that "probably each of the characters, protein, crude fat, and ash content, segregates as a definite and distinct unit character".

Lindstrom and Gerhardt (80) found that dent and sweet varieties of corn were sharply differentiated in the chemical constitution of their seed, especially in relative amounts of sugars, dextrins, starches and fat. This fact, together with our knowledge

of the mechanism of heredity in this species, provides an excellent background for an exact determination of the mode of inheritance of these chemical characters. Another advantage in the genetic analysis lies in the precise, cellular mechanism of double fertilization in the embryo sac whereby the endosperm tissue is developed as the result of a triple fusion of nuclei, two from the female and one from the male parent. This nuclear behavior permits a detailed comparison of the influence of one, two or three doses of genetic factors for any endosperm characters, including the chemical characters involved in these investigations. A thorough genetic study of endosperm factors controlling aleurone and endosperm color has demonstrated beyond reasonable doubt that their inheritance is typically Mendelian in nature. It becomes of immediate interest to determine whether chemical characters, from the quantitative standpoint, obey the rules of inheritance as do the qualitative characters of the endosperms noted above.

In the original or parental types used (80), sweet corn had nearly twice as much fat as dent corn when the entire kernel was used for analysis. When only the endosperm was used, this same sweet corn had nearly three times as much fat. This, of course, indicates that in dent corn most but not all the fat resides in the embryo, whereas in sweet corn a much larger proportion actually exists in the endosperm itself. This relation is also consistently shown in the various hybrid generations of this cross. Calculations show that approximately 74% of the total fat content in dent corn resides in the embryo, the remainder presumably being in the endosperm tissue, particularly in the aleurone layer of cells.

In sweet corn kernels, more of the fat was contained in the endosperm tissue. In these kernels approximately 64% of the fat was in the germ, leaving 36% for the endosperm and pericarp tissues.

The F_1 kernels of the sweet corn × dent corn cross distinctly show an intermediate condition with a slight dominance of the lower fat percentage, which is consistently true whether the entire kernel or only the endosperm tissue be considered. Pearl and Bartlett (100) found the same general situation in their crossbred or F_1 kernels, using only the entire kernel for analysis.

Segregation of high and low fat values in the F_2 and backcross generations is very evident. The extracted sweet corn in all cases

is practically as high or higher in fat than in the original variety. This is true both in the entire kernel and in the germless grains. Presumably there is a direct association of fat values with the carbohydrate situation. There is, however, no apparent cumulative effect in fat values which might be expected if this association were complete.

In all the crosses reported in these investigations, the parental varieties of dent or sweet corn carried different endosperm colors, one or the other being yellow. Because of this relation it is possible to determine any association of endosperm color with high or low sugar, starch or fat content, whether this association be due to genetic linkage or to chemical or physiological correlation. Pearl and Bartlett's (100) data suggested a slight correlation between these characters. In their F_2 generation from a Yellow dent × White sweet corn cross, there seemed to be a higher sugar content in the yellow F_2 seeds than in the white. Conversely the white seeds showed a higher fat value. This finding was confirmed by Lindstrom and Gerhardt and was traced to a genetic linkage.

Soy beans—oil percentage. Varieties of soy bean tested at the Delaware Agricultural Experiment Station showed considerable range in time of maturity, size of plant, coarseness or fineness of plant parts, and yield of seed (32). Some varieties stand erect, others have the tips of the branches slightly twining. Varieties exhibit considerable differences in the habit of bearing their branches near the ground. The color of seeds may be black, yellow, olive, green, brown or mottled. Size of the seed also varies widely. In some varieties 35 seeds will weigh 10 grams, in others 140 seeds are required. The period of maturity ranges from 90 to 135 days. Seventeen varieties of the fifty-one analyzed contained 40% protein. The difference in protein content ranged from 35% to 44.8%, while the average for the whole number was 39.2%. The oil or fat content varied from 14.1% to 20.4% with an average of 18%. Only four varieties produced more than 20% of oil.

In determining the varietal differences in the oil content of soy bean seed, Garner, Allard and Foubert (29) grew a number of varieties under uniform conditions, used the purest seed obtainable, and repeated the tests for several seasons. Their data show

that there are enormous varietal differences in soy beans both as to size of seed and as to oil content. Furthermore, it should be noted that the seasonal effects of the three years did not influence the several varieties alike with respect to either of these two characters. More extensive tests through a period of five years and with several additional varieties fully confirmed these results. It is clear, therefore, that in soy beans heredity is a very important factor, not only with respect to the size and the oil content of the seed but also as regards the extent to which these characters respond to change in environment.

Soy beans—iodine values. One of the important uses for soy bean oil is as a substitute for linseed oil in the manufacture of paint and varnish. Its value for this purpose depends upon the amount of unsaturated acid contained. It follows that in a study of the factors affecting the oil content, it becomes of interest to learn what effect, if any, such factors have upon the iodine number which is taken as an index of the degree of unsaturation.

Stark (113) found that wide variations may exist in the composition (protein and oil content) of the same variety when grown in different localities of Illinois. Such variations as were observed in this investigation were apparently not due to geographical position or climatic conditions, but may probably be attributed chiefly to differences in soil fertility and soil reaction. Of the four varieties there was a greater difference in iodine value within one variety than existed between the other three e.g., Black Eyebrow averaged 123.2, Manchu 131.6, Midwest 131.0, and Wilson Five 136.3. The differences within the varieties were, respectively, 5.1, 5.0, 7.4 and 3.7.

Cotton—oil percentage. When different varieties of cotton were grown under uniform conditions, using the purest seed obtainable and repeating the tests several seasons, marked varietal differences in size of seed and other important characters were noted, but the percentage of oil remained remarkably constant when the environmental conditions were the same (29). Williams (120) obtained somewhat greater variations in oil content in a test with 21 varieties.

Grape seed—iodine values. Investigations on grape seed oils indicate that the variety of the vine has a much greater influence on the composition of the oil than the region in which it is grown

(14). Analysis of 19 samples of oil prepared in the laboratory by extraction with trichlorethane and hot filtration through charcoal, from seed of known origin, gave the following minimum, average, and maximum results, respectively: yield 7.1, 15.2, 20.0%; specific gravity at 15° C., 0.924, 0.0933, 0.950; viscosity at 35° (in dynes per sq. cm.) 0.2723, 0.3500, 0.4237; viscosity at 57°, 0.1342, 0.1800, 0.2423; acidity (as oleic acid) 0.24, 0.73, 1.80%; refractive index 1.4723, 1.4767, 1.4797; saponification number 190.5, 197.6, 208.0; iodine number (Hanus) 115.3, 126.4, 132.6; acid number (Andra) 12.8, 20.7, 29.3.

The number of carbon atoms in glycerides. It is significant that all fatty acids in fats contain an even number of carbon atoms, and that those of 18 carbon atoms (C_{18}) predominate, the carbon chain being a multiple of the carbohydrate unit six (C_6).

The three main fatty acids. While there is a difference in the relative amounts of different acids in different oils, there seems to be little difference in the nature of these acids, these usually being oleic, linoleic and linolenic.

In the case of oleic acid, for which a number of isomerides are possible with different positions of the double linking, the natural acid is practically always the Δ^9 acid, that is, it is the one with the double bond between the ninth and tenth carbon atoms, beginning with the carbon of the carboxyl (COOH) group. The existence of this acid is doubtless related to its activity, since the activity varies considerably with the position of the double linking in the carbon chain. This may show that while an organism may, in the course of evolution, acquire the ability to produce new substances, that which it had been producing in its primitive state it continues to produce in exactly the same form. The particular oleic acid found in plants must have been formed in the early stages of the evolution of the vegetable kingdom (103).

An examination of the relation between the value of the iodine number and the nature of the unsaturated acids contained in different vegetable oils shows that a low value of this number does not indicate the exact nature of these acids. In some cases the degree of unsaturation is due to a small amount of highly unsaturated acids, while in others it is due to a large amount of more nearly saturated acids. In most cases, the high unsaturation corresponded to the predominance of linoleic acid. This was proved by an examination

of the amount of crystalline tetrabromide and hexabromide obtained from the saponified oils (103).

ALIPHATIC ACIDS SPECIFIC TO FAMILIES

Seed fats from plants belonging to the same or closely allied families often contain the same fatty acids, and several families are characterized chemically in that one or more fatty acids predominate in the oils of all the species.   The literature shows that such relationships have been recognized for a long time, and the names of the individual fatty acids indicate the plant families: Lauraceae—lauric acid; Myristicaceae—myristic acid; Linaceae—linoleic acid, linolenic acid.   In a recent detailed analysis (38) of the mixed fatty acids of various seed fats from some of the Palmae, Cruciferae and Umbelliferae, together with earlier data on other members of these families and of the Myristicaceae, the fatty acids have been compared in detail.   In any of these four families the composition of the fatty acids of the seed fats is of the same general type, but each family is marked by definite and specific characteristics in the composition of the fatty acids.   Thus the Palmae seed fats almost always contain 46%–50% of combined lauric acid, with minor amounts of caprylic, capric, myristic and palmitic acids, and relatively small proportions of oleic acid; in the Myristicaceae seed fats myristic acid predominates; in cruciferous seeds there is usually 40%–50% of combined erucic acid, the remainder consisting of oleic and linoleic acids in varying proportions; and umbelliferous seeds appear to be characterized by an isomeric form of oleic acid, petroselinic acid (20%–75%), which has not yet been observed in the seed fats of any other family except the closely related Araliaceae. Both the Umbelliferae and the Araliaceae belong to the order Umbelliflorae.   Other characteristic acids are the cyclic acid, chaulmoogric group, of the Flacourtiaceae (114).   And then there are the "carriers" of specific color reactions, as the chromogen of the Halphen reaction in the oils of the Malvales.   It is inferred that the seed fats of any botanical family have certain characteristics of their own and much in common with each other, differing from those of dissimilar families.   This is not universal, however; in the Euphorbiaceae, for example, castor oil from seeds of *Ricinus communis* is entirely different from the oils of the genera *Aleurites* and *Mercurialis*.   The predominance of one acid in the oils from a

family is confined only to the seed or kernel oils, and the oil or fat from other parts of the plant may be entirely different; *e.g.,* palm oil from the fruit pulp of the oil palm contains no lauric acid. Hilditch has devised a useful method for separating the glycerides in the oils, and therefore for determining the exact manner in which the fatty acids are grouped together in the glycerides. This work has shown that in the seed fats there is often a similarity, even between the actual glycerides present in closely related plants, and also that there tends to be an even distribution of the various fatty acids throughout the glycerides. Hence in the seed fats mixed glycerides are the rule, and simple glycerides occur only when one fatty acid predominates to such an extent as to render their formation unavoidable, *e.g.,* trimyristin in nutmeg fat. This, however, does not hold for the glycerides in parts of the plant other than the seed, *e.g.,* in fruit pulp or leaves; these resemble animal fats in that there is no tendency toward an even distribution of the fatty acids, and therefore simple glycerides are more common (39). Again, in seeds storing fat in both embryo and endosperm, the composition of the fat may be quite different in the two tissues.

INCREASE IN NUMBER OF C-ATOMS IN ACIDS AND NUMBER OF ACIDS
    IN SEED FATS WITH ADVANCE IN EVOLUTIONARY POSITION

In the latest compilation of analyses of seed fats (39*a*), data from 16 natural orders (24) are given. When the component acids of the families of these orders was considered (88) it was found that seven orders had an increase in the number of acids, eight had an equal number of acids and one had a decrease in the number of acids with an advance in evolutionary position of their constituent families.

When the number of carbon atoms of these acids was considered (88) it was found that eight orders had an increase in the number of C-atoms, six had an equal number of C-atoms and two had a decrease in the number of C-atoms with an advance in evolutionary position. If, however, the terminal families of those analyzed of the Malvales, Myrtiflorae, Contortae and Tubiflorae (*i.e.,* respectively, Sterculiaceae, Myrtaceae, Asclepiadaceae and Acanthaceae) be removed from consideration, then three of these four orders showed an increase in the number of acids and all four showed an increase in the number of C-atoms in these acids with an increase

in evolution. An increase in the number of C-atoms indicated in these instances an increase in molecular weight of the acids which contain them.

RANGE AND AMPLITUDE OF IODINE VALUES IN SPECIES, GENERA AND FAMILIES

The properties of a vegetable oil of any plant in any part of the world can be foretold from two factors, its place in the botanical system and its climatic source. By determination of the iodine number different species of the same genus grown under similar climatic conditions showed a similar content of unsaturated fatty acids in the fatty oils derived from them (64). Experiments conducted to study the influence of climate on the composition of fatty oils showed that oils containing glycerides of unsaturated acids with one double bond (oleic, erucic or ricinic) are indifferent toward climatic changes, while in acids with three double bonds (α- and β-linolenic acid) the iodine number decreased with increase of temperature or with growth of the plant in more southerly regions. Oils with two double bonds (of the linoleic acid type) hold an intermediate position.

As a criterion, the iodine number may be used, since it is easy to determine and indicates changes of composition usually with sufficient clarity. For example, for linseed oils in the region of Moscow during the period of 1907–1916, Ivanov (58) found the iodine number to be 180 ± 4; for sunflower of Veron, the iodine number for a 25-year period averaged 125 ± 3; for the region about Cuba, in the case of the same oil, the number was 122 ± 3. The relative constancy of the composition applies—for a constant ecological factor—not only for oils of the same botanical variety, but also—though to a rather limited extent—to fats of allied species of the same genus, as tabulated on the following page.

The iodine number of a species can be approximately foretold by a knowledge of the climate in which it lives and the genus to which it belongs. For instance, the place of *Prunus divaricata* (alytascha) is well established in the genus *Prunus,* and geographically it is found in the Caucasus at 1.6–1.8 km. above sea level. These data fix the properties of its oil; it must resemble almond oil, but on account of the higher altitude of its habitat its percentage of unsaturated acids must be greater. The actual results of analysis of

AVERAGE VARIATIONS OF THE IODINE NUMBERS OF OILS OF PLANTS OF THE
SAME GENUS

Genus	Family	No. of species studied	Vicinity of cultivation	Iodine No. (Range of variation)
Malva	Malvaceae	5	Leningrad	124 ± 4
Hibiscus	"	4	Moscow	121 ± 5
Gossypium	"	3	Taschkent	108.7 ± 5
Lavatera	"	6	Turkestan & Cuba	120 ± 8
Trollius	Ranunculaceae	3	Moscow	139 ± 5
Aconitum	"	9	"	115 ± 7
Delphinium	"	6	"	112.3 ± 8
Thalictrum	"	7	"	174 ± 12
Aquilegia	"	5	"	191.4 ± 13

seven samples of oil from kernels of alytascha confirm this con-
clusion (64).

The maximum range in iodine numbers for the oils of families
may also be established. For instance, the published analyses of
the oils from members of the Anacardiaceae lead to the conclusion
that this family yields oils containing principally the glycerides of
oleic acid, and any linoleic glyceride that is present decreases in
amount from the subtropic toward the tropic climate (64). The
iodine number of members of the Anacardiaceae can not be below
25 nor above 120.

The oils in all members of most of the smaller families are in
close agreement with each other, as is shown in the Cruciferae,
Cucurbitaceae, Lauraceae, Myristicaceae, Pinaceae, Polygalaceae,
Rutaceae, Sapindaceae, Sapotaceae, Simarubaceae, Solanaceae and
Umbelliferae.

In the large families the oils of the different genera are usually
in close agreement when grouped according to tribes. For instance
(84):

Leguminosae[1]
    Subfamily I. Mimosoideae: (F) *Parkia*, (ND) *Pentaclethra*
    Subfamily II. Caesalpinoideae: (SD) *Caesalpinia*
    Subfamily III. Papilionatae:
        Tribe 3, Genisteae: (SD) *Cytisus*, (SD) *Spartium*, (ND) *Lupinus*
        Tribe 4, Trifolieae: (ND) *Trigonella*, (ND) *Trifolium*, (ND)
            Ornithopus, (ND) *Melilotus*, (ND) *Medicago*
        Tribe 5, Loteae: (ND) *Lotus*, (ND) *Anthyllis*
        Tribe 6, Galegeae: (ND) *Galega*, (D) *Robinia*, (D) *Caragana*, (D)
            Amorpha
        Tribe 7, Hedysareae: (ND) *Arachis*, (ND) *Onobrychis*

Tribe 8, Vicieae: (SD) *Vicia*, (SD) *Cicer*, (SD) *Pisum*, (SD)
    Lens
Tribe 9, Phaseoleae: (SD) *Voandzeia*, (SD) *Cajanus*, (SD)
    Dolichos, (SD) *Canavalia*, (SD) *Mucuna urens*, (SD) *Vigna*,
    (SD) *Phaseolus Mungo*, (SD) *Phaseolus lunatus*, (SD)
    Phaseolus inamoenus, (SD) *Phaseolus coccineus*, (SD) *Pha-*
    seolus vulgaris, (D) *Glycine*
Tribe 10, Dalbergieae: (F) *Dipteryx*, (F) *Pongamia*

Gramineae
Tribe 1, Paniceae: (D) *Panicum*
Tribe 2, Maydeae: (SD) *Zea*
Tribe 3, Oryzeae: (ND) *Oryza*
Tribe 6, Andropogoneae: (SD) *Sorghum*
Tribe 7, Phalarideae: (SD) *Phalaris*
Tribe 12, Hordeae: (SD) *Secale*, (SD) *Triticum*, (SD) *Hordeum*

Cucurbitaceae
Tribe 1, Cumerineae: (F) *Hodgsonia*, (ND) *Telfairia*, (F) *Luffa*,
    (SD) *Acanthosicyus*, (SD) *Cucumis*, (SD) *Citrullus*, (SD)
    Cucurbita, (SD) *Bryonia*
Tribe 3, Elaterieae: (SD) *Echinocystis*

Guttiferae
Tribe 2, Moronobeae: (F) *Symphonia*, (F) *Pentadesma*
Tribe 3, Garcinieae: (F) *Garcinia*
Tribe 4, Calophylleae: (ND) *Calophyllum*, (ND) *Mesua*

ADVANCE IN PHYLOGENETIC POSITION IN THE CRYPTOGAMS AS INDICATED BY THEIR FATS

It has already been determined that seed fats of tropical angio-
sperm families have lower iodine values (85.3), lower molecular
weights and higher melting points than the seed fats of temperate
angiosperm families (124.0). It has likewise been shown that when
seed fats of these families are first separated according to climate of
habitat, the higher the plant family is in evolutionary position the
more likely it is to form seed fats with large iodine numbers, higher
molecular weights and lower melting points. There are also indi-
cations of an increase in the number of acids in seed fats with ad-
vance in evolutionary position (83, 86, 88, 89).

It is of interest, therefore, to find experimental evidence in favor
of a similar situation in the lower plants. In Table II five organ-
isms are listed in evolutionary sequence. From the figures it is
apparent that the fats with the highest iodine values are produced
at the lowest temperature, and in *Aspergillus niger* the fat produced
at a temperature intermediate between the highest and the lowest
has also an intermediate iodine value. The temperature and mean
iodine values are, respectively: for I. Timothy grass bacillus 14°

[1] The abbreviations used are as follows: D, drying oil; SD, semi-drying
oil; ND, non-drying oil; F, fat.

TABLE II

Plant	Temperature (degrees C.)	Reaction time (days)	Molecular weight (mean)	Iodine number of fat
I. Division, Schizophyta,				
Timothy grass bacillus	14°	∷	∷	57–59 (116)
	35°			31–35 (116)
XI. Division, Eumycetes				
1. Class, Phycomycetes				
Rhizopus nigricans	12°	30	289	87.2–88.7
				(mean 88) (101)
	25°	10	288	79 (101)
		13	287	77.3
				(mean 78) (101)
2. Class, Ascomycetes				
Aspergillus fischeri	20°	16	∷	93 (104)
	37°	12	∷	88 (104)
Aspergillus niger	18°	17	302	146.7–153.6 (101)
		56	323–330	145.9–150.2
				(mean 149) (101)
	25°	10	287	124 (101)
		14	304	132.5 (101)
		17	290	131.3 (101)
		35	287	127 (101)
				(mean 129) (101)
	35°	6	293	92.1 (101)
		7	285	92.3 (101)
		9	290	99.9
				(mean 95) (101)
4. Class, Basidiomycetes				
Sterigmatocystis nigra	17°	∷	∷	112–116 (116)
	35°			83–87 (116)

(iod. val. 58), 35° (33); for II. *Rhizopus nigricans* 12° (88), 25° (78); for III. *Aspergillus fischeri* 20° (93), 37° (88); for IV. *A. niger* 18° (149), 25° (129), 35° (95); and for V. *Sterigmatocystis nigra* 17° (114), 35° (85).

There are also indications that the fats with the highest iodine values and highest molecular weights are also produced by the plants highest in evolution when these plants are grown at comparative temperatures, *e.g.*, I. 14° (iod. val. 58), II. 12° (88) (mol. wt. 289), III. 20° (93), IV. 18° (149) (m.w. 323), and V. 17° (114) or I. 35° (33), II. 35° (78) (m.w. 287), III. 37° (88), IV. 35° (95) (m.w. 290) and V. 35° (85). Conversely, as shown by the above figures, there are indications that the fats with the lowest iodine values and lowest molecular weights are produced by the plants lowest in evolution when these plants are grown at comparative temperatures.

EVOLUTIONARY STATUS OF SPERMATOPHYTE FAMILIES IN RELATION TO THEIR FATS

Some 318 fats have been analyzed from 83 spermatophyte families. As there are 295 such families, about 30% of them have thus been analyzed (86). The families that produced these materials were divided into climatic groups as follows: tropical, tropical-subtropical, subtropical, subtropical-temperate, temperate and widely distributed. By far the most of these families were in the tropical, temperate, or widely distributed groups. The tropical and temperate families that contain fatty oils were tabulated in (86). These two zones, tropical and temperate, were chosen for discussion because they represented extremes in climatic difference.

The tabulated list of tropical fat families begins with the Palmae and Araceae and closes with the Rubiaceae and Cucurbitaceae, and includes many quite evenly dispersed families between these limits. For example, there were two families with botanical serial numbers below 1000, three between 1000 and 2000, eight between 2000 and 3000, four between 3000 and 4000, ten between 4000 and 5000, fifteen between 5000 and 6000, eight between 6000 and 7000, five between 7000 and 8000, and two between 8000 and 9000 (17). Consequently the group presented a representative cross section of all tropical families.

These dispersals of data were graphically portrayed in the scatter

diagram (Fig. 3 in 86) and showed that any additional data will fall within the scope of the statistics already obtained.

From these tabulated data it was definitely determined that there was a consistent variation in some of the chemical and physical properties of these materials according to the tropical and temperate climates in which they were produced. For instance, it was found that the tropical and subtropical fats had higher melting points and lower iodine values (*i.e.*, they were more saturated) than the fatty oils of temperate climates (83). This finding was supported by the evidence of Hilditch (38) who, in analyzing fat constituents, found specific acids for four plant families, namely lauric (mol. wt. 200, m.p. 48° C.) for the Palmae, myristic (mol. wt. 228, m.p. 58° C.) for the Myristicaceae, erucic (mol. wt. 338, m.p. 33.5° C.) for the Cruciferae, and petrosilinic (mol. wt. 282, m.p. 14° C.) for the Umbelliferae. As the Palmae and Myristicaceae are tropical and the Cruciferae and Umbelliferae temperate, the average molecular weight of the tropical families, 214, is lower than that of the temperate, 310. At the same time the average melting point of these tropical acids, 53°, is higher than that of the temperate, 23°.

NATURAL OR EVOLUTIONARY SYSTEMS OF PLANTS

Several systems of plant classification have been developed, such as those of Bentham and Hooker (8), Engler and Prantl (24), Bessey (10), Rendle (106), Hutchinson (43) and Mez (95). These systems vary in the arrangement of their families, not only as to the relative positions of the families within the systems, but also as to the families chosen for origin and termini. They all agree, however, in having more primitive families as origins and more highly organized families as termini. For convenience, Engler and Prantl's system was chosen to illustrate the relative general evolutionary position of the plant families in respect to the chemical compounds formed by them. In this paper the serial numbers given these families by DeDalla Torre and Harms (17) were used.

In giving serial numbers to the various plant families in the Engler and Prantl system, DeDalla Torre and Harms begin with the most primitive plants and continue to the most highly organized. The arrangement of plant families in accordance with their evolutionary positions assumes a tree-like form with the more primitive

families at the base of the tree and the most highly organized families at the tips of the branches, the topmost families on the tree representing the most highly evolved plants. It might appear at first glance that a linear system of numbering would not truly represent the proper relative positions of the various families in their order of evolution. Such, however, is not the case, as the following examples will show. Gymnosperms are treated first and have smaller numbers than angiosperms. In angiosperms the monocotyledons have lower numbers than the dicotyledons. In the dicotyledons the members of the subdivision of Archichlamydeae have lower numbers than have the Metachlamydeae. Of the Metachlamydeae the order Diapensiales is succeeded by the Ebenales, Contortae, Tubiflorae, Rubiales and Cucurbitales.

Each order has the most primitive family as its lowest number and the latest family has the highest number. In the DeDalla Torre and Harms numbering of the various Engler and Prantl genera it is as though the evolutionary tree were stripped of its branches and each branch, beginning with the lowest on the tree, were laid tip to base in a horizontal line which terminates with the highest branch.

In order for evolution to have taken place in a tree-like form, it is necessary that a lower branch be older (more primitive) than the one above it. Consequently each branch base represents a stage in progressive development which can be correctly expressed by serial numbers. Likewise the apical tips of the branches typify steps in the evolutionary progress of the plant families which also can generally be accurately shown with respect to each other by serial numbers. It may be, however, that the tip of a lower branch portrays a higher stage than the base of the branch next above it. If such is the case, it is nevertheless true that this higher stage is not higher than the base of the branch to which the tip belongs. Therefore it is shown that the average serial number of a lower branch must generally be lower than the average serial number of the branch just above it. Consequently, although the serial numbers of DeDalla Torre and Harms constitute a linear system, they are nevertheless sufficiently representative of the tree-like natural system of plant evolution for our purpose. The position of the orders on Figure 3 (in 86) showed this to be true. Should an individual family be wrongly placed in the system, use of the average botanical number of groups of families tends to eliminate such error.

EVOLUTIONARY STATUS OF FAMILIES IN RELATION TO THE
IODINE VALUES OF THEIR FATS

In table 2 (in 86) the temperate fatty oils were separated from those produced by tropical plant families. It is apparent from this table that temperate fatty oils have higher average iodine values (124.0) than tropical (85.3).

In Figure 3 (in 86) the data of the tropical fats from Table 2 (in 86) were arranged in a scatter diagram. Each point represented the average iodine number of the fats obtained from a certain family, which family was indicated by its numerical position in the Engler and Prantl system. A vertical line connecting various points showed the families of an order.

To determine the trend of these points, a straight line was used. A straight line was chosen because it constitutes the best means of showing a trend. There was only one straight line which fitted most accurately the plotted data. The constants of this line of best fit were determined by the method of least squares (96). It was assumed that the botanical numbers were free from any error; therefore, they were used as independent variables. The resulting equation was

$$y = 78.98834 + 0.00013287x$$

where x indicates the botanical numbers and y the average iodine numbers of the fats. This equation had a positive slope (+ 0.00013287) which showed the line to have an upward trend.

If both the botanical numbers and the molecular weight were considered as not free from error, the trend would have had a similar nature.

It can be definitely stated, therefore, that the higher the tropical plant family is in evolutionary development, the greater will be its tendency to produce fats of large average iodine numbers (and also the lower will be their melting points).

ONTOGENY VERSUS PHYLOGENY IN RELATION TO FAT FORMATION
AND PERCENTAGE

It is now a well known fact that in the formative development of seed, carbohydrate is changed to a highly saturated fatty oil and this fatty oil is in turn changed to a less saturated fatty oil. If chemical ontogeny is a recapitulation of chemical phylogeny then

one might apply this test to the monocotyledons and dicotyledons to determine which group originated first. Such a test might also be applied to the Archichlamydeae and Sympetalae.

The taxonomic distribution of oil and starch in seeds has already been tabulated (85). These data were reclassified (Table III) as to the percentages of starchy and oily embryos in the seeds of Monocotyledonae, Archichlamydeae and Sympetalae. The percentages of starchy and oily embryos in these groups were also determined as well as the nature of the total seed contents.

An examination of the data in Table IV showed a definite increase in the percentages of families with oily embryos, oily albumen and oily general contents in the series beginning with the monocotyledons, through the Archichlamydeae to the Sympetalae (91). There was a corresponding decrease in the starchy contents. The monocotyledons also had less saturated seed oils than those of the Archichlamydeae and Sympetalae (87). From these results it was deduced that the monocotyledons in their present chemical development are more primitive than the dicotyledons. Therefore the monocotyledons may have originated before the dicotyledons or as an early branch from the primitive dicotyledons. As a result of a similar deduction it is stated that the Archichlamydeae are more primitive chemically at least than the Sympetalae.

Use of Chemical Factors in the Study of Phylogeny. The iodine values of seed fats have been used in the comparison of plant families and orders as well as in the comparison of different plant groups, *e.g.,* the monocotyledons with the dicotyledons. It has already been shown (86), first, that the more closely plants are related, the more similar are their chemical products; and second that the more highly evolved the plant (according to the Engler and Gilg (23) classification) the larger are the iodine values of its fats, provided the plants grow in the same climate. These findings have been made use of in further comparisons of angiosperm phylogeny.

The arrangement of families and orders in the Bessey system is to be found in Bessey (9), and the arrangement of families and orders in the Engler and Gilg system was taken from Engler and Gilg (23).

In the discussion of results obtained in the tables, the first column (in Tables III to VI inclusive in 87) includes all the tropical families from which data have been obtained, while the other columns

TABLE III

Percentages of Families That Have Starchy or Oily Embryos, Albumen, and General Contents

Plant group	Embryos		Albumen		General contents oily %
	Starchy %	Oily %	Starchy %	Oily %	
Monocotyledons	4/33 = 12	15/33 = 45	19/33 = 57	9/33 = 27	5/33 = 15
Archichlamydeae	21/137 = 15	62/137 = 45	23/137 = 16	43/137 = 30	69/137 = 50
Sympetalae	4/42 = 9	18/42 = 42	1/42 = 2	17/42 = 40	23/42 = 54

include only part of these families. Therefore the left hand column gives a better average value founded on a larger group of plant families than any of the other columns. The figures in it can consequently be considered as more representative and dependable than those in the other columns.

Are the Magnoliaceae pre-Ranunculaceaen? Hallier (36, 37) believed that the Ranunculaceae and Nymphaeaceae were descended from the Magnoliaceae through the Schizandraceae, Lardizabal-aceae and Berberidaceae. Engler, however, has concluded that it is not likely that such characteristically woody families as the Mag-noliaceae and Lauraceae have arisen from herbaceous ancestors, or *vice versa,* but that these woody types have had a quite different origin from the herbaceous Ranunculaceae, as have most of the monocotyledons whose protangiospermous ancestors may be as-sumed to have been herbaceous (12).

Fats have been found in the Ranunculaceae, Berberidaceae, Lardi-zabalaceae. All three of these families are found for the most part in the temperate zone, and consequently they can be successfully compared chemically. Table I (in 87) shows that the iodine values of the fats diminish from the Ranunculaceae to the Lardizabalaceae. Consequently, Hallier's theory of descent has chemical support as far as chemical data are available.

The Ranunculaceae consist mainly of herbs, the Berberidaceae of herbs and shrubs, and the Lardizabalaceae of shrubs. Here there is an indication that shrubs may precede herbs phylogenetically.

The Magnoliaceae are mainly a sub-tropical family and there-fore can not be compared chemically with the three temperate zone families considered here. The average iodine number of the fats of the Magnoliaceae, 95.5, should be lower than the temperate fats and higher than the tropical fats in order to comply with the general rule. It was assumed that the Magnoliaceae are plants of the temperate zone as are the three other families; their low value, 95.5, therefore would indicate that they are more primitive than the Berberidaceae and Ranunculaceae.

Are Herbs Derived from Trees? The foregoing chemical re-sults indicate that herbs may be derived from trees. Eames (21), in a paper devoted to the subject, brought forward evidence that the earliest dicotyledons possessed a solid tubular woody cylinder of considerable thickness which has gradually been reduced and finally

broken up into a circle of separate strands, characteristic of the "typical" herbaceous condition. Such an hypothesis of reduction from primitive arborescent forms has also been worked out under the direction of E. C. Jeffrey by several other members of his laboratory (2, 4, 70). In more recent papers (109, 110) there is evidence in support of this view from palaeobotany, phylogeny, anatomy and geographical distribution. It is no wonder, then, that Bessey (10) included in his "General principles adopted for the classification of plants" the postulate that "in certain groups, trees and shrubs are probably more primitive than herbs".

This hypothesis has been considered from the standpoint of the chemical products derived from plants. In Table II (in 87) the fats from tropical plant families were considered in this respect.

From the final average obtained of the iodine values of fats, there is a clear indication that trees produce fats of lower iodine values than shrubs, and that shrub fats have lower iodine values than those of herbs. There is chemical support, therefore, for the contention of Bessey (10), Sinnott and Bailey (109), and others, that angiosperm herbs have been derived from woody plants.

Do Dicotyledons Precede Monocotyledons? In the history of the development of taxonomic systems, there is a difference of opinion among authors as to the precedence of monocotyledons or dicotyledons in the natural system. John Ray (105), de Jussieu (74), Eichler (22), Engler and Prantl (24), Rendle (106) and Johnson (72) considered monocotyledons as the antecedents of dicotyledons. The following botanists have believed dicotyledons to be the forbears of monocotyledons: de Candolle (13), Bentham and Hooker (8), Wettstein (119), Bessey (10), Hallier (36, 37), Clements (15), Jeffrey (71), Hutchinson (43).

Bessey's (10) phylogenetic postulate is that dicotyledons are more primitive in origin than monocotyledons. Chemical results indicate, however, that monocotyledons preceded dicotyledons or may have been an early branch from the dicotyledons or may have had a separate origin. In Table III (in 87) the iodine values of fats are higher in dicotyledons than in monocotyledons, being 85.85, 109.40 and 77.92 in the dicotyledons, and 63.62, 80.98 and 28.90 in the monocotyledons.

Families in the monocotyledons and dicotyledons were given according to the Engler and Prantl system. The chemical data used

were from McNair (86). The families of the monocotyledons and dicotyledons were grouped according to the predominance in them of herbs, shrubs and trees; herbs; herbs and shrubs; shrubs and trees; and trees.

Polypetaly versus Gamopetaly. In his consideration of the flower types, Bessey (10) considers that "free petals (polypetaly) are more primitive than connate petals (gamopetaly)". The Bessey system divides both the Axiflorae and Calyciflorae into Apopetalae (Polypetalae) and Gamopetalae. But in the Engler and Prantl classification the Apopetalae are found in the Archichlamydeae and the Gamopetalae in the Metachlamydeae.

This postulate of Bessey has found support in the chemical products of the Polypetalae and Gamopetalae. For instance, the fats (Table IV in 87) 82.57 vs. 84.40 and 95.19 vs. 110.79. In Engler and Prantl's system the Archichlamydeae (Apopetalae) are according to both phylogeny and chemical products more primitive than the Metachlamydeae (Gamopetalae). This was shown by the fat iodine values (Table IV in 87) 82.94 vs. 93.84, 76.77 vs. 86.82.

Polycarpy versus Oligocarpy. Bessey (10) advanced another postulate which has found chemical evidence in its favor. This postulate is that "many carpels (polycarpy) preceded few carpels (oligocarpy)".

Bessey separates the Polycarpellatae from the Dicarpellatae in his Gamopetalae. Chemical evidence for these divisions in the Bessey system was found in the fats (Table V in 87). However, the fats do not support the Bessey theory as shown by Table V: 88.04 vs. 83.74 and 88.04 vs. 59.20.

Apocarpy versus Syncarpy. In his consideration of the evolution of flower structure, Bessey (10) states: "free carpels (apocarpy) are more primitive and from them connate carpels resulted; sometimes, however, when the carpels have remained loosely united during the evolution, they may again become quite free".

In Bessey's classification the Apopetalae have separate or united carpels, but the Gamopetalae have united carpels only. Consequently, as was shown above under "Polypetaly versus Gamopetaly", chemical evidence indicates that free carpels are more primitive (Table VI in 87).

The Engler and Gilg system makes a clear separation of apocarpy from syncarpy in the Ranales (apocarpy) and Rhoeadales (syn-

carpy). The iodine values of the fats give favorable evidence to the theory, e.g., (Table VI in 87) 72.03 vs. 105.95 and 72.86 vs. 92.40.

Engler and Gilg System from a Chemical Standpoint. The various divisions under the Engler and Gilg system of classification were divided for the purpose of this study into the following various sections such as the monocotyledons: A, the Amentiferae (from the Piperales to Urticales); B, Proteales to Polygonales; C, Centrospermae; D, Ranales to Umbelliflorae; A, Diapensiales to Plumbaginales; B, Ebenales; C, Contortae; and D, Tubiflorae to Campanulatae. These divisions or sections were considered as radii originating from a central point, thus forming a cart wheel design. This central point was taken as zero, and the positions of the various orders on the radii were taken as equal to the chemical results obtained from a study of the iodine values of their fats. If one looked down upon a tree from its top the branches would appear to radiate from a common center. In a similar way the branches of the phylogenetic tree were presented.

The iodine values of the fats (Fig. 1 in 87) of tropical families and sections of the Engler and Gilg system show, in general, a close agreement with the botanical classification. Complete agreement is found in the Monocotyledonae, Amentiferae and Metachlamydeae.

According to the Engler and Gilg phylogenetic system, the Santalales are farther advanced than the Proteales. The iodine values, however, indicated the reverse to be true. Inasmuch as the chemical differences between them was very slight (Proteales 85 and Santalales 83) the chemical evidence was hardly sufficient to counteract the morphological evidence and phylogenetic position.

Likewise the chemical evidence presented is probably too insignificant to warrant serious consideration for departure in phylogenetic position from Engler and Gilg classification, namely, that the Parietales 70.57 should phylogenetically precede the Ranales 72.03, or that the Geraniales 65.82 should precede the Ranales 72.03, or that the Sapindales 79.24 should precede the Malvales 90.65, or that the Umbelliflorae 91.8 should precede the Myrtiflorae 100.08.

The Bessey System from a Chemical Standpoint. The Bessey system (9), like that of Engler and Gilg, recognizes the two main

divisions of monocotyledons and dicotyledons. The dicotyledons, however, are divided differently than in the Engler and Gilg system. Bessey divides them into Axiflorae and Calyciflorae. In the Axiflorae, "axis flowers", the axis of the flower is normally cylindrical, spherical, hemispherical or flattened, bearing on its surface the hypogynous perianth, stamens and carpels (or the stamens may be attached to the corolla). In the Calyciflorae, "cup flowers", the axis of the flower is normally expanded into a disk or cup, bearing on its margin the perianth and stamens (or the latter may be attached to the corolla). The Axiflorae are considered as more primitive.

The groups of the Bessey system were considered as radii originating from a central point. This central point was taken as zero and the positions of the various orders on the radii as the various chemical results obtained from a study of their fats.

The iodine values of the fats (Fig. 2 in 87) of the tropical families and sections of the Bessey system showed in general a close agreement with the botanical classification. Only three of the orders did not agree with the botanical classification, *viz.*, the Caryophyllales, Celastrales and Ranales. The Caryophyllales 105 should precede the Ebenales 87. The Celastrales 110 should precede the Umbellales 92. The Ranales 80 should be nearer the point of origin than any of the other dicotyledons.

DISCUSSION OF THE ENGLER AND GILG AND BESSEY SYSTEMS FROM A CHEMICAL POINT OF VIEW

The Engler and Gilg system considers that monocotyledons precede dicotyledons. The Bessey system on the contrary classes dicotyledons before monocotyledons. The chemical evidence in regard to the iodine values of the fats (as given in 87) supports the Engler and Gilg contention: that monocotyledons may have preceded dicotyledons (Table III in 87).

With this exception both systems agree in their use of the following phylogenetic principles:

a) Polypetalous flowers are more primitive than gamopetalous flowers.

b) Numerous carpels represent a more primitive condition than few carpels.

c) Separate carpels represent a more primitive condition than united carpels.

All of these, except *b*, have been shown to hold true from the chemical investigations. In this case chemical evidence (Table V in 87) indicated that few carpels may represent a more primitive condition than many carpels. The Bessey, and the Engler and Gilg systems, however, differ in the relative importance given to these phylogenetic principles.

In both systems the Ranales did not fall in the position allotted to them. There was, however, better agreement with their position in the Engler and Gilg system than with their position in the Bessey system.

The Caryophyllales and Gentianales are far removed in the Engler and Gilg but may agree better with that classification than with Bessey's (Fig. 1 in 87).

The Celastrales and Umbellales did not harmonize with the Bessey system (Fig. 2 in 87). These were in better accord in Engler and Gilg.

SUMMARY

The oil content of seed varies with the successive stages of maturity. During ripening there is an increase in the amount of unsaturated acids of drying oils, whereas the composition of non-drying oils may remain practically the same.

Maximum oil production may be reached and further production cease when chlorophyll disappears. However, increase in the iodine value may continue after disappearance of the chlorophyll.

Saturated fatty acids are formed first but are progressively transformed into acids with a greater degree of unsaturation.

Considering only percentage of oil, there is a very sharp increase during the first few weeks after blossoming in soy beans, and then only a slow gain until near the end of ripening. During the final stage of ripening there is a decrease in both the size of the seed and in oil content.

The iodine number increases quickly at the last stage of maturity, there being little difference in the early stages.

In sunflower oil, the iodine value was found to remain almost constant during ripening, but the saturated acids decreased steadily in amount while the unsaturated acids increased in amount.

In ripening seeds increase in the percentage of oil is accompanied by a decrease in the carbohydrate percentage.

The total percentage and iodine value of oil is affected more by the character than the length of the growing season.

Vigorously vegetative plants have lower concentrations of fat in their leaves than weakly vegetative plants.

Different varieties are not always influenced in the same manner by environment. Percentages of oil produced are influenced by both heredity and environment.

The comparative effect of soil types on per cent of oil formation varies with seasonal conditions. In some seasons clay soil produces a higher percentage of oil than loam, and *vice versa*.

Under practical conditions climate is a more potent factor than soil in modifying the oil content of seed.

Bacteria have been found to produce the most fat when in neutral media and the least when the media are acid or alkaline.

Increased amounts of fat are formed by mold when grown in neutral or slightly alkaline media, a high concentration of glucose, a low concentration of ammonium nitrate and at high temperatures.

Within the limits ordinarily met in farm practice the relative fertility of the soil does not greatly influence size of the (soy bean) seeds or oil content. Increased applications of nitrogen lowered considerably the oil content of the kernels. Increased applications of phosphorus and potassium had no appreciable effect.

Additions of phosphorus to pot cultures of soy beans resulted in increased oil content, but addition of potassium alone gave practically no increase in oil content.

In seedling cultures of *Picea*, potassium-free cultures gave stronger reactions for fats in the root systems. When phosphorus was omitted fats were absent.

Linseed oil had a higher iodine value when produced on non-fertilized fields.

More saturated fats are produced by fungi at lower temperatures.

Lower saturated acids are first formed and higher unsaturated acids are formed later.

Mild warm climate favors formation of saturated fatty acids and of unsaturated acids with one double bond. Cold climate favors formation of unsaturated acids with two or three double bonds.

Starch in evergreen leaves (*Linnaea*) forms at moderately elevated temperatures and is reconverted into oil when the temperature is gradually lowered.

As plants grow nearer to the equator the calorific value and the iodine value of their fats decrease. This also occurs in transplanted plants.

Oil from the heartwood of deodar has an increased iodine value when the trees are grown at a higher altitude. This is also true of the fats of the seeds of many plants.

Fats in British brown algae (Phaeophyceae) show an increase in amount and in iodine value with an increase in depth of immersion.

Shade increased the starch content and decreased the oil content of flax seed. Heavy shade lowered the iodine value.

Increased moisture content of the soil has been found to increase the total oil in flax seeds. The iodine value is likewise lowered by deficient soil moisture.

In seed germination absorption of water is accompanied by diminution in the amount of oil in the seed.

As in many higher plants, so in yeast: the equilibrium carbohydrate ⇌ fat is controlled by variations in water content.

Dense sowing of flax was found to increase the iodine value of oil.

In corn kernels crude fat segregates as a definite and distinct unit character and is inherited in accordance with Mendelian principles.

There are varietal differences in soy beans as to per cent of oil content as well as in iodine value.

In cotton the percentage of oil is hereditable but is greatly influenced by change in environment.

In grape seed oil the variety of the vine has a much greater influence on the composition of the oil than the region in which it is grown. This is shown in per cent and iodine value.

All fatty acids in fats contain an even number of carbon atoms.

Seed fats from plants belonging to the same or closely allied families often contain the same fatty acids, and several families are characterized chemically in that one or more fatty acids predominate in the oils of all species.

When the component acids of the families of 16 natural orders are considered, it is found that seven orders have an increase in the number of acids, eight have an equal number of acids and one has a decrease in the number of acids with an advance in evolutionary position of their constituent families.

When the number of carbon atoms of these acids is considered it is found that eight orders have an increase in the number of C-atoms, six have an equal number of C-atoms, and two have a decrease in the number of C-atoms with an advance in evolutionary position.

The properties of a seed oil of any plant in any part of the world can be foretold from two factors, its place in the botanical system and its climatic source. Different species of the same genus grown under similar climatic conditions show a similar content of unsaturated fatty acids.

The maximum range and amplitude in iodine values for the oils of families, genera and species may also be established. There is an increase in both range and amplitude with increase in size of the taxonomic group.

The oils of most of the smaller families are in close intrafamilial agreement, while those of larger families are often in better agreement if considered in tribal groups.

There is close agreement between the oils of species of a genus.

From the study of iodine values of cryptogam fats it has been found that fats with highest iodine values are produced at the lowest temperature, and that fats with the highest iodine values and highest molecular weights are produced by plants highest in evolution when grown at comparable temperatures, and *vice versa*.

From a study of iodine values of seed fats of spermatophyte families it can be definitely stated that the higher the tropical plant family is in evolutionary development the greater will be its tendency to produce fats of large iodine values (and also the lower will be their melting points).

In the formative development of seed, carbohydrate is changed to a highly saturated fatty oil and this fatty oil is in turn changed to a less saturated fatty oil. If chemical ontogeny is a recapitulation of chemical phylogeny, then applying the above chemical process, monocotyledons in their present chemical development are more primitive than dicotyledons, and the Archichlamydeae are more primitive chemically at least than the Sympetalae.

By applying the two facts (a) that the more closely plants are related, the more closely similar are their chemical products, and (b) that the more highly evolved the plants the larger are the iodine values of their seed fats provided the plants grow in the same climate, the following phylogenetic deductions have been made:

The Magnoliaceae are more primitive than the Berberidaceae and Ranunculaceae.

Angiosperm herbs may have been derived from trees.

Monocotyledons are more primitive than dicotyledons.

Free petals (polypetaly) are more primitive than connate petals (gamopetaly).

Few carpels (oligocarpy) may have preceded many carpels (polycarpy).

Free carpels (apocarpy) are more primitive than connate carpels.

In a chemical comparison of Engler and Gilg's classification with Bessey's it is apparent that in both systems the Ranales do not fall in the position allotted to them but are in better agreement with their position in the Engler and Gilg system.

The Ebenales and Gentianales are out of harmony in the Bessey system, but in better agreement in the Engler and Gilg system (both in Contortae).

The Caryophyllales and Gentianales of Bessey are far removed in Engler and Gilg (being, respectively, Myrtiflorae and Contortae), and the chemical findings may agree better with that classification than with Bessey's.

The Celastrales and Umbellales do not harmonize with the Bessey system. These are in better accord with Engler and Gilg where the orders are considered as Sapindales and Umbelliflorae.

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GENE SEGREGATION IN AUTOTETRAPLOIDS

THOMAS M. LITTLE

U. S. Dept. Agr., Bureau of Plant Industry

The genetics of autotetraploids is complicated by various factors which are not encountered in the genetics of diploids. The most important of these complicating factors lies in the fact that one of the fundamental concepts underlying diploid genetics, that of "purity of gametes", is meaningless in the discussion of tetraploid genetics. In a tetraploid the gametes may be hybrid as well as "pure". This leads to a further complication in the zygotes, namely, that instead of having only one type of heterozygote, as in the diploid, we have three, which along with the two homozygotes gives five possible genotypes involving any one pair of genetic factors. These five genotypes are known as quadriplex, triplex, duplex, simplex, and nulliplex, according to whether there are four, three, two, one, or no dominant genes present.

Another complicating factor in the study of tetraploid segregation is the relation between certain cytological phenomena and the genetic ratios obtained. Diploid ratios involving a single gene are affected little or not at all by such variable factors as pairing, multivalent formation, non-disjunction, chiasmata, and the position of the gene on the chromosome in relation to the centromere, yet all of these factors have a profound influence upon tetraploid ratios. These points will be discussed in more detail later, but it should be pointed out here that while it is well recognized that variations in cytological behavior introduce variables in tetraploid segregation which make a precise mathematical analysis of the ratios difficult or even impossible, still it is felt that certain advantages are to be gained in a study of such ratios. The mere fact that tetraploid ratios are affected by cytological behavior opens up the possibility of studying the relationships between certain cytological and genetic phenomena, which cannot be studied in diploids where genetic ratios are, in general, more stable.

The literature on the segregation of genetic characters in autotetraploids falls into two main categories, those papers dealing with the theoretical aspects of the problem and those in which actual experimental data are presented and interpreted. While some of

the published work deals with both aspects, it has seemed most convenient to discuss these two categories separately, first treating the development of the theories concerning autotetraploid segregation, then reviewing the published data in the light of these theories.

REVIEW OF LITERATURE

Theories of Autotetraploid Segregation

The first report on segregation in autotetraploids appeared when Gregory (37) presented data on the tetraploid *Primula sinensis*. He attempted to explain his results on the basis of duplicate-factor ratios commonly found in diploids. Thus he assumed that the ratios he obtained on selfing a tetraploid hybrid corresponded to the 15:1 ratios found in diploids, and that the backcross ratios were 3:1. His conclusions were based on the false assumption that pairing must always take place between a chromosome derived from the maternal parent and one from the paternal parent (allosyndesis). Muller (61) pointed out this fallacy and assumed that pairing took place between any two of the four homologous chromosomes at random, independently of their origin. On this basis he concluded that the ratio obtained from selfing a duplex heterozygote should be 35:1, and that backcrossing such a hybrid to a homozygous recessive tetraploid should give a 5:1 ratio. Moreover, he attempted to show that Gregory's data fit these ratios better than those which Gregory himself had assumed.

No further theoretical treatment of the problem appeared until Haldane (38) published generalized formulae covering all types of polyploids. For autotetraploids he showed that, if one assumed random assortment of chromatids rather than of chromosomes, a duplex hybrid would give a ratio of 187:9 (or approximately 21:1) on selfing, and 11:3 on backcrossing to a homozygous recessive. These ratios have attracted considerable attention by subsequent authors, and data which seem to fit them are usually referred to as displaying "chromatid segregation". Along with this term, the term "chromosome segregation" has come into general use, referring to ratios which seem to fit more closely those given earlier by Muller. The unsoundness of considering these two types of segregation as separate and distinct fixed ratios, and of assigning observed data to one or the other, was brought out by Mather (59). However, the

practice has persisted to a certain extent to the present time. This point will be discussed later in more detail.

Mather published three papers (57, 58, 59) dealing in part with segregation in autotetraploids. These papers discussed the subject in the light of cytological facts, many of which were not well understood at the time the previous papers were published. In referring to the terms "random chromatid segregation" and "random chromosome segregation", he states: "It has been recognized by all later workers that these segregation . . . expectancies are in the nature of limiting types, and that the true segregations to be expected from autotetraploid organisms will actually lie somewhere between them. . ." .

Further on in the same paper he makes this somewhat contradictory but probably sounder statement: "A new segregation was established (57) for the case of completely equational separation, and random chromatid segregation was shown to be the result of a combination of the two types in the random proportions of 1/7 reductional and 6/7 equational separation".

Thus the first statement refers to random chromatid segregation as a limiting type, while the second statement represents it as an intermediate type. This brings out an interesting contrast between the phenomenon of linkage of genes in diploids and linkage with the centromere in tetraploids, which has never been emphasized. In diploids the upper limit of crossing-over between two linked genes is 50%, and this limit is the equivalent of independent assortment or random segregation. In tetraploids, the upper limit of crossing-over between a gene and the centromere is likewise 50%, but this is not equivalent to random chromatid segregation. Instead, the ratios calculated on the basis of random chromatid segregation are equivalent to those which would be obtained if the amount of crossing-over between the gene and the centromere were approximately 43%.

One of Mather's most important contributions was the formulation of methods of calculating indices which could be used to characterize an observed ratio in terms of cytological behavior. These indices were based on the products of the amount of genetical non-disjunction in a quadrivalent (a), times the amount of equational separation of chromatids (e). The latter variable is in turn dependent upon the frequency of chiasmata, and hence the per cent

of crossing-over between the centromere and the gene in question. It was at first maintained (59) that the values for these variables would be the same in triplex and simplex hybrids, but would be different in duplex hybrids, where change of pairing partners might alter their value. Therefore the index calculated from simplex and triplex data was designated as alpha, while the one calculated from duplex data was called beta. However, this view has recently

TABLE I

AUTOTETRAPLOID RATIOS EXPECTED ON THE BASIS OF VARIOUS HYPOTHESES

Mating	Gregory	Muller	Haldane	Mather	Mather's ratios when alpha is 1/3
AAAa selfed	inf. 0	inf. 0	783 1	$64-a^2$ a^2	575 1
AAAa × AAaa	inf. 0	inf. 0	389 3	$48-a-a^2$ $a+a^2$	107 1
AAAa × Aaaa	inf. 0	inf. 0	769 15	$64-4a-a^2$ $4a+a^2$	563 13
AAAa × aaaa	inf. 0	inf. 0	27 1	$8-a$ a	23 1
AAaa selfed	15 1	35 1	187* 9	$35-2a-a^2$ $1+2a+a^2$	77 4
AAaa × Aaaa	7 1	11 1	347 45	$44-5a-a^2$ $4+5a+a^2$	95 13
AAaa × aaaa	3 1	5 1	11 3	$5-a$ $1+a$	7 2
Aaaa selfed	3 1	3 1	559 225	$48-8a-a^2$ $16+8a+a^2$	407 169
Aaaa × aaaa	1 1	1 1	13 15	$4-a$ $4+a$	11 13

* Frequently called a 21 : 1 ratio.

been corrected (35), and it is now considered that one index will suffice to specify the segregations from all three types of tetraploid heterozygotes. The lower limit of this index should be zero, when there is no crossing-over between the gene and the centromere, or when no quadrivalents are formed. The upper limit should be 1/3 when there is 50% crossing-over between the gene and the centromere and random disjunction of quadrivalents.

The expected ratios based on the four hypotheses of Gregory, Muller, Haldane, and Mather are summarized in Table I, the last column giving the ratios expected on the basis of Mather's hypoth-

esis when alpha has its maximum value of 1/3.   It will be observed
that there are nine types of crosses in tetraploids capable of giving
segregating progenies, in contrast to diploids where there are only
two such crosses, namely, the heterozygote times itself and the
heterozygote times the homozygous recessive.

Experimental Data

   Primula.   The first experimental data on tetraploid segregation
were published by Gregory (37), reporting on work with the tetra-
ploid form of *Primula sinensis.*   One of the pairs of factors upon
which he reported was the *Thrum* (short-style) versus *Pin* (long-
style) pair, designated by him as $A:a$ but referred to in the later
literature as $S:s.$ Data were presented involving four different
mating combinations, namely, duplex and simplex plants selfed and
each of these backcrossed to the nulliplex.   When the data are com-
pared with the expectancies based on his own hypothesis and the
hypotheses of Muller and Haldane, the P values obtained are 0.27,
0.21 and 0.08, respectively.   Due to the small populations involved,
therefore, the data did not differ significantly from any of these
three theoretical ratios.   The other pair of factors studied was green
versus red stigmas.   It can also be shown that the data on this
character did not deviate significantly from any of the three theoreti-
cal ratios.   Thus, much of the discussion as to which ratios these
data represented was statistically meaningless.

   Further data on *Primula sinensis* appeared in an extensive report
by Sömme (77) who reported on the inheritance of seven pairs of
factors, including the two discussed by Gregory.   The conclusion
was reached that none of the ratios obtained differed significantly
from those postulated by Muller.

   De Winton and Haldane (23) published results on three pairs
of factors in *Primula,* including the S and G factors discussed by the
two earlier authors and the $B:b$ factor pair for magenta versus red
flower color.   Particular attention was paid to linkage among these
factors, since it had been shown in diploids that all three are located
on the same chromosome.   It was concluded that all three of these
factors were located near the centromere, so that double reduction
was disregarded.   While there was a rather consistent excess of
recessives in their data (as would be expected with double reduc-
tion), they attempted to explain part of these excesses by assuming

that some of the parents were pentasomic for the chromosome involved. However, in a later report on linkage in diploids (24), the data from tetraploid segregation were used to locate the position of the centromere on the linkage map of this chromosome. They stated that the data indicated that the centromere was close to the loci of the S and B factors but further distant from the G locus.

Datura. Blakeslee, Belling, and Farnham (9) published a report on the segregation of two factor pairs in tetraploid *Datura.* The value of these data, which lies in the extremely large populations involved, has apparently never been fully appreciated. Since no one has ever published a statistical analysis of the data, a comparison is included here between the observed ratios and the expectancies according to the Muller hypothesis (which the authors accepted), the Haldane random chromatid ratios, and the Mather ratios obtained by calculating alpha. In the case of the *purple : white* factor pair, a chi-square test gives a P value of only 0.012 when applied to the Muller hypothesis and less than 0.01 when applied to the Haldane ratios. On the other hand, when expectancies are calculated on the basis of Mather's alpha and compared with the observed data, a P value of 0.475 is obtained. Similar results are obtained from data on the *armed : inermis* factor pair, where both the Muller and Haldane ratios give P values of less than 0.01 while the Mather ratios give a P value of 0.15. Data from crosses involving triplex plants are not included in these chi-square tests, since the number of recessives expected from such crosses is too small. However, it should be noted that a total of 14 recessives appeared in populations where none was expected according to the Muller hypothesis.

Potato. In recent years the evidence indicating that the cultivated potato is an autotetraploid has created considerable interest in the genetics of this plant. The cytological evidence is conflicting in regard to the nature of polyploidy in the potato (13, 36, 29, 60). While the earlier workers were inclined to class this plant as an allotetraploid, some of the more recent writers, especially Cadman (13), favor the view that it is an autotetraploid. Meurman and Rancken (60) consider that it is fundamentally autotetraploid but have observed that there are only two instead of four chromosomes with satellites, indicating that some chromosome differentiation has taken place, so that the potato now has some of the characteristics of allotetraploids.

The genetic evidence as to the nature of polyploidy is likewise conflicting. All reports on the genetics of potatoes up to 1930 interpreted the inheritance as disomic. Müller (62) was the first to explain data on a tetrasomic basis, in connection with studies on resistance to dry rot. All of Lunden's and Jørstad and Lunden's earlier works (40, 53, 54, 56) interpreted the inheritance of genetic factors on a disomic basis, but in a more extensive study (55), based on very large populations, Lunden concluded that the segregation of all factors studied was tetrasomic. Some of these data fit the Muller ratios very closely, but in several cases there was an excess of recessives due to double reduction, and one factor gave segregations which were shown to fit the Haldane random chromatid ratios better than the Muller ratios. Since Mather's index of double reduction had not yet been published, no attempt was made to characterize the segregations on this basis.

Since Lunden's work there has been a tendency among most investigators to explain their results on a tetrasomic rather than a disomic basis. Krantz and his co-workers have explained the inheritance of pollen sterility on an autotetraploid basis (41). In their studies on resistance to common scab (42) they showed that the plants fell into five classes as regards breeding behavior, corresponding to the five possible genotypes in an autotetraploid. Stevenson, Schultz, and Clark (81) gave a tetrasomic explanation for the inheritance of immunity to Virus X. Cadman (12) has thoroughly reviewed the subject of autotetraploid inheritance in the potato and presented further evidence based on segregation of differences in reaction to virus inoculation. He has been the only worker up to the present time to employ Mather's formulae for obtaining an index of double reduction in potatoes.

The early papers by Black (5, 6, 7) all gave a disomic interpretation to the data on inheritance, but in his most recent paper (8) he has come to the conclusion that the potato is an octoploid rather than an allo- or autotetraploid.

The exact nature of polyploidy in the potato is a problem that is still far from being solved, by either cytological or genetic investigations. It is doubtless complicated by the fact that this species is a very old polyploid which has undergone considerable change since it first arose. It is highly probable that some of the chromosomes have become differentiated into two pairs so that their be-

havior is like that of an allotetraploid, while others have undergone little or no differentiation and continue to pair among themselves at random like the four homologous chromosomes of an autotetraploid.

Tomato. The first artificially induced tetraploid to be studied genetically was in the tomato. Sansome (73) investigated the inheritance of seven characters and concluded that three of them displayed "chromatid segregation", one displayed "chromosome segregation", one was intermediate, and in the other two the populations were not large enough to decide. As a matter of fact, the data indicate that with probably all of the factors, segregation was intermediate, as should be the case. When Mather later published his papers giving methods for calculating indices of double reduction, he drew on some unpublished data of Sansome for use in illustrative examples. In a cytological study of tetraploid tomatoes, Upcott (84) found that the amount of quadrivalent formation was clearly compatible with Sansome's genetic results.

Lindstrom (50) made a new approach to the study of quantitative genes of tomato by comparing their genetic behavior in tetraploids and diploids.

Dahlia. Cytological and genetic studies on the garden dahlia (43, 44, 45) have led Lawrence to conclude that *Dahlia variabilis* is an allo-octoploid resulting from doubling the chromosome number in a hybrid between two tetraploid species. At first he believed that some of the genetic factors displayed tetrasomic inheritance, while with others the inheritance was disomic. He assumed that the Y factor for flavone formation was of the former type, while I, also a flavone factor, was of the latter type. However, in a later study with Scott-Moncrieff (45) it was concluded that the I gene was also inherited tetrasomically, but that simplex plants resembled the nulliplex, thus modifying the expected tetrasomic ratios.

It was in Lawrence's first report on *Dahlia* (43) that the phenomenon of double reduction was first suggested.

Rubus. Tetraploid segregation in *Rubus* was reported by Crane and Darlington (15) in connection with the factors for presence and absence of prickles. They found an excess of recessives over those expected according to Muller's hypothesis, which they explained on the assumption that there was a "tendency toward allosyndesis". This explanation was later abandoned (16) and their data were explained on the basis of chromatid segregation, but the

populations were too small to permit any statistical conclusions other than that the deviations from Muller's ratios were significant.

The type of segregation observed in several tetraploid species of *Rubus* has been used by Crane (14) and Thomas (83) to determine whether these species were allo- or autotetraploids.

Maize. Randolph (70), in a report on the cytogenetics of tetraploid *Zea mays,* states that segregations approximating 35:1 ratios were noted for several factors. However, he says that the "data are inadequate to determine whether or not there are significant deviations from the expected ratios".

Lotus. An interesting case of tetraploid segregation in *Lotus corniculatus* was reported by Dawson (22). This species is tetraploid, and two genetic types are found in natural populations, differing only in that one produces hydrogen cyanide and the other does not. It was found that this cyanogenetic character gave ratios which fit very closely those of Muller's random chromosome segregation, and it was therefore concluded that this species is an autotetraploid. The cytological observations gave no evidence of its autotetraploid nature, since formation of quadrivalents was very rare, a condition which is usually assumed to indicate allotetraploidy. The failure of quadrivalent formation would account for the lack of double reduction found in the observed ratios.

Lythrum. The anomalous behavior of heterostyly in *Lythrum salicaria* has been the subject of controversy since first described by Barlow (2) over thirty years ago. East (25, 26, 27, 28) attempted to explain his observations on the basis of linked duplicate factors, both of which were lethal in the homozygous dominant state. However, the breeding behavior of the mid-style form observed by Fisher and Mather (32, 33, 34, 35) could not be explained by East's hypothesis, and their results were explained on a polysomic basis. The chromosome number of this species is not definitely known, since different numbers have been reported by various workers. Nevertheless, it is known that the species is polyploid in relation to the basic number of the genus, and there are indications that it is numerically a hexaploid. So far it has not been possible to determine whether the inheritance of mid style is tetrasomic or hexasomic, but further work is being carried out to determine this point. Evidence of double reduction was obtained in the segregations for this factor, and a rough estimate of the index of double reduction was calculated by Fisher and Mather (35).

Antirrhinum. In some unpublished work by the writer (52), the segregation of four genetic factors was studied in colchicine-induced tetraploids of the snapdragon, *Antirrhinum majus.* The diploid behavior of these four factors had been studied by Baur (3, 4) and Onslow (65, 66) and found to be independently inherited. Moreover, three of the factors could be read in the seedling stage, so that it was possible to grow and classify very large populations. In the tetraploids it was found that the segregation of each of these four factors was different, depending upon the amount of double reduction due to crossing-over between the gene and the centromere. It was possible to show that the segregation of all factors gave a significant excess of recessives over the expectancies based on the Muller random chromosome hypothesis. Furthermore, the ratios obtained with the anthocyanin-modifying factor (A) also differed significantly from the Haldane random chromatid ratios.

Hybrids between tetraploids of different varieties within this species have been referred to by Sparrow, Ruttle, and Nebel (78, 79) as allotetraploids, because they are usually more fertile than intra-varietal tetraploids. Cytological observations, however, did not reveal any marked differences in meiotic behavior between inter- and intra-varietal tetraploids. These observations, along with the fact that genetic factors show typical autotetraploid segregations, would seem to indicate that increased fertility found in some inter-varietal tetraploids is due to genetic factors for fertility rather than to selective pairing which ordinarily distinguished allotetraploids from autotetraploids.

DISCUSSION

Conditions Necessary for Genetic Analysis of Tetraploids

In spite of the fact that a large number of tetraploids have been discovered or produced artificially, many of them are not amenable to genetic analysis, due chiefly to three difficulties commonly encountered. The first and most common of these difficulties is the high degree of sterility often found in autotetraploids. The very nature of tetraploid segregation requires that very large populations be grown and analyzed in order to obtain data that will have any statistical significance. In general, larger populations are required in order to draw valid conclusions in tetraploid material than in diploids. The high degree of sterility of many tetraploids makes it

impossible or at least extremely difficult to secure such large populations.

The second difficulty, curiously enough, is most often found when the first difficulty is absent. It consists of the amphidiploid-like behavior found in some tetraploids. It is now generally recognized that "autotetraploidy" and "amphidiploidy" are rather arbitrary terms and that many tetraploids are intermediate in their behavior so that the distinction is not always clear-cut. Amphidiploidy is not confined to species hybrids, but may arise in autotetraploids through structural changes in the chromosomes or some other form of chromosome differentiation. Increased fertility and decreased segregation are simultaneous consequences of a change from auto-tetraploidy to amphidiploidy, so that many of the most fertile tetraploids display little or no segregation.

The third difficulty is the lack, in many species, of clear-cut genetic characters which lend themselves readily to classification and analysis. A prominent example of this is found in species of *Lilium*. Nearly all genetic factors which distinguish varieties within a species, such as *L. longiflorum,* are quantitative in nature, and a study of their inheritance would be extremely difficult even in diploids. Another example is that of scab-resistance in the potato, which Krantz and Eide (42) found very difficult to classify with any high degree of precision.

Cytological Behavior and Tetraploid Segregation

As stated previously, cytological behavior has a more pronounced effect on genetic segregation in tetraploids than in diploids. There are three main variables in cytological behavior which may affect the proportion of recessives found in a segregating progeny. These will be discussed in the following order: first, mode of pairing; second, quadrivalent formation; and third, chiasma frequency.

Pairing in a tetraploid may be classified as either random or selective with reference to any given group of four homologous chromosomes. Even within the same tetraploid, some members of a genom may undergo random pairing, while others may display varying degrees of selective pairing. In random pairing, any one of the four homologous chromosomes may pair with any one of the other three with equal probability. The hypotheses of Muller, Haldane, and Mather all assume random pairing, but Gregory's hypothesis assumes a special type of selective pairing.

Selective pairing occurs when the four homologs are not equally homologous but tend to fall into two groups such that the two chromosomes within a group display more affinity than two chromosomes from different groups. In other words, they possess differential affinity. Homology is a relative term and can vary from absolute identity, such as is found in doubled haploids, to the very weak homology found in secondary pairing. As a result the pairing in tetraploids can vary from completely random where the four chromosomes are equally homologous, to completely selective where the plant behaves functionally as a diploid. All degrees of selective pairing between these two extremes may exist. To illustrate how selective pairing may affect segregation in autotetraploids, let us assume that we have a duplex hybrid with the constitution $AAaa$. Furthermore, let us designate the two similar pairs of chromosomes carrying these genes with subscript 1 and 2. Then in a plant with the constitution $A_1A_1a_2a_2$, if selective pairing is complete, there will be no segregation, since only gametes with the constitution A_1a_2 can be formed, and these will unite to form progeny with the constitution $A_1A_1a_2a_2$. If selective pairing is not complete, so that A_1 occasionally pairs with a_2, then gametes can be produced with the constitution A_1A_1 or a_2a_2, and the latter type may unite to give a homozygous recessive segregate. This is known to occur in *Primula kewensis* (63, 67) where a limited amount of segregation takes place. It was also reported by Skalinska (76) in an allotetraploid specimen of *Aquilegia*. Chromosome doubling in a diploid hybrid A_1a_2, or crossing of two autotetraploids $A_1A_1A_1A_1$ and $a_2a_2a_2a_2$, will give rise to amphidiploids with the constitution $A_1A_1a_2a_2$ just discussed. However, under some conditions we may have tetraploids with the constitution $A_1a_1A_2a_2$. In this case, complete selective pairing will produce gametes with the constitution A_1A_2, A_1a_2, a_1A_2, and a_1a_2 in equal proportions, and the phenotypic ratios on selfing will be 15A : 1a. Moreover, under these conditions two types of duplex hybrids will be produced, those like the parent and non-segregating types $A_1A_1a_2a_2$ or $a_1a_1A_2A_2$. This type of segregation is found in diploids when duplicate factors are involved, and its occurrence is sometimes considered an indication of tetraploid ancestry. We have already pointed out that newly arisen tetraploid hybrids are almost certain to have the constitution $A_1A_1a_2a_2$. The origin of the second type, $A_1a_1A_2a_2$, is probably secondary, re-

sulting from the occasional pairing and consequent crossing-over between A_1 and a_2 chromosomes in such a way that there is an exchange of alleles A and a without conspicuously altering the original differentiation of the chromosomes. This is borne out by the fact that practically all cases of duplicate factor ratios are found in plants that, on other grounds, show evidence of being old polyploids.

Gregory's hypothesis was based on the assumption that there was complete selective pairing between chromosomes from opposite parents. This type of pairing was later referred to as "allosyndesis", and Crane and Darlington (15) spoke of deviations from the 35:1 ratio in *Rubus* as due to a "tendency toward allosyndesis", an explanation which they later retracted (16). The terms "autosyndesis" and "allosyndesis" have been purposely avoided in the foregoing discussion to avoid the confusion that is common in their usage. Autosyndesis is generally defined as pairing between chromosomes derived from the same parent, and allosyndesis as pairing between chromosomes from opposite parents. However, it makes a difference whether we refer to the original parents or to the immediate parents. Darlington (21, p. 200) states: "The distinction between the two is only relative to the immediate parents . . .". He further restricts the use of the term to "pairing of dissimilar chromosomes". Hence he says: "Autosyndesis occurs in allopolyploids under the following conditions: In the normal polyploid species as an exceptional occurrence". Cadman (12), on the contrary, speaks of "duplex allotetraploids . . . where strict autosyndesis prevails". Unlike Darlington, he is referring to autosyndesis as pairing between chromosomes from one of the *original* parents of the allotetraploid. Sharp (74, 75) uses the terms in this latter sense, although he refers to them as "auto-" and "allosynapsis". The terms are used in still another sense in the literature when they refer to genetic differences. Thus in a duplex hybrid $AAaa$, pairing between A and A or a and a is sometimes called "autosyndesis", while pairing between A and a is called "allosyndesis". Lindstrom (51) has used the terms in this genetic sense.

It appears that selective pairing is rare or entirely absent in most of the recently originated autotetraploids that have been studied genetically, such as *Primula sinensis, Datura, Lycopersicon esculentum* and *Antirrhinum*. The genetic ratios obtained in these plants are therefore not affected by this cytological variable. However, in

old polyploids such as potato and *Dahlia*, there is apparently some selective pairing which tends to alter the segregation of certain genetic factors.

The second cytological variable that affects segregation is the formation of quadrivalents. Quadrivalent formation involves pairing among all four homologous chromosomes. Hence, Gregory's ratio, based on pairing only between chromosomes of opposite parents, could not be obtained where quadrivalent formation is common. Muller's ratios, on the other hand, are not affected by the presence or absence of quadrivalents as long as crossing-over does not occur between the gene and the centromere. In fact, his ratios can be obtained in the complete absence of quadrivalent formation. This appears to be the case in *Lotus corniculatus* (22). Haldane's ratios can not be obtained unless the chromosomes involved form quadrivalents regularly. In Mather's formulae, the quantity *a*, representing the amount of genetical non-disjunction, is dependent upon the amount of quadrivalent formation, attaining its random value of 1/3 only if quadrivalents are regularly formed.

The third cytological variable is the frequency and position of chiasmata formation. Since separation at first division is always reductional at the centromere, equational separation at a given locus depends upon the formation of a chiasma between that locus and the centromere. In Mather's formulae, the amount of equational separation is designated as *e*. If one chiasma is always formed between the centromere and the locus in question, separation at that locus is always equational and *e* has the value of 1. Stated in terms of cross-over percentage, this would be equivalent to fifty per cent crossing-over, and in general terms, *e* is twice the cross-over percentage. Many *Drosophila* workers have shown that temperature changes and age affect the amount of crossing-over (10, 68, 69, 80). Ernst (31) has shown that sudden changes in temperature reduce the chiasma frequency and hence the amount of crossing-over in *Antirrhinum*. The subject of environmental effects on chiasma formation has been reviewed by Oehlkers (64). Since the segregation in a tetraploid varies with the amount of chiasma formation, and this is in turn influenced by environmental factors, tetraploid ratios should be expected to vary according to the environmental conditions which prevailed when the parent organism was undergoing meiosis. This has never been demonstrated, but would be well worth investigation.

In a tetraploid, when the two chromosomes in a gamete are derived from sister chromatids, double reduction is said to have occurred. Since double reduction tends to increase the proportion of homozygous gametes (both recessive and dominant), it affects the genetic ratios by increasing the proportion of recessive phenotypes above that expected on the assumption that the chromosomes behave as units and do not undergo double reduction. Hence, any cytological behavior that favors double reduction tends to increase the proportion of recessives in a ratio. In order for double reduction to take place, a special set of cytological conditions must be satisfied. First, paired chromosomes bearing opposite alleles must pass to the same pole at the first division. This is termed "genetic non-disjunction" and its frequency was designated by Mather with the symbol a. Genetic non-disjunction is in turn dependent upon the formation of trivalents or quadrivalents, since with bivalents the two members of the pair almost invariably pass to opposite poles. The second condition which must be fulfilled is that at least one chiasma must be formed between the centromere and the locus of the gene in question, resulting in equational separation of the gene loci at the first division. When both of these conditions are fulfilled, two chromosomes pass to the same pole at the first division, each consisting of two chromatids bearing opposite alleles. At the second division, when the centromeres divide and pass to opposite poles, the two chromatids bearing the same allele may pass to the same pole, thus resulting in double reduction. Inasmuch as double reduction depends upon these two conditions, its frequency may be expressed as the product of the frequency of genetic non-disjunction a, and the frequency of equational separation e. This product ae is designated by Mather as $alpha$.

The index $alpha$ gives us the product of the amount of genetical non-disjunction and the amount of equational separation, but tells us nothing about the relative part that each of these variables plays in affecting segregation. The value of a will vary from zero, when no quadrivalents are formed, to 1/3, the random value obtained when the chromosomes always associate in quadrivalents. The value e, on the other hand, can vary from zero to one as the cross-over percentage between the gene and the centromere varies from zero to fifty per cent. Thus the value of $alpha$ should vary from zero to 0.33. If we observe cytologically that the frequency of

quadrivalents is very high, we can assume that the value of a approaches its upper limit of 1/3, and as a result gain an approximation of e by multiplying the value of $alpha$ by three.

We have seen what relationship exists between tetraploid segregation and several specific aspects of cytological behavior. It should also be pointed out that segregation in tetraploids has an important bearing on several general cytological concepts. One of these is in regard to the time of crossing-over. Deviations from the Muller ratios furnish us with genetic proof that crossing-over takes place in the four-strand stage between two of the strands. The first demonstration of this fact was furnished by Anderson (1) in connection with studies on crossing-over in attached X-chromosomes of *Drosophila*. It was further demonstrated by Bridges and Anderson (11) in the X-chromosomes of triploid females of *Drosophila*. It is an interesting fact that the data on tetraploid segregation published previously by Blakeslee, Belling, and Farnham (9) can now be interpreted to prove the same fact. The appearance of recessive progeny from triplex hybrids could not have taken place without double reduction and hence crossing-over in the four-strand stage. The first genetic demonstration of double-strand crossing-over in *Zea* was made by Rhoades (71, 72) in work based on the genotypic constitution of trisomic types. Emerson and Rhoades (30) have pointed out the significance of chromatid or double-strand crossing-over in relation to the upper limit of recombination. Finally, the ingenious work of Lindegren on *Neurospora* (48, 49) proved the occurrence of double-strand crossing-over by an entirely different method and shed light on further details in the behavior of chromatids and chromosomes during meiosis.

Another general problem, upon which tetraploid segregation can throw light, is the old problem of reductional and equational separation of chromosomes during meiosis. It was long thought that the first division of meiosis was "reductional" or "heterotypic" while the second division was "equational" or "homotypic". This distinction is now known to apply only to the centromere and closely adjacent regions of the chromosomes, for the other portions of the chromosomes may divide equationally at the first division as the result of crossing-over. As a result of these "equational exceptions", double reduction can occur, a fact first suggested by Lawrence (43) and further discussed by Darlington (20) in 1929. This problem has been discussed in detail by Mather (57, 59).

Genetical Behavior and Tetraploid Segregation

Not only does tetraploid segregation have a close relation to various cytological problems, but it is also of interest in connection with certain genetical problems. The foremost of these is the location of the centromere on the linkage map of a chromosome. It is impossible to solve this problem in the study of diploid ratios (except in the fungi), but a solution can be approximated if we have autotetraploids from which to secure data on segregation. This method was used by De Winton and Haldane (24) to determine the approximate position of the centromere on the linkage map of one of the chromosomes of *Primula sinensis*. As already pointed out, there is no method of determining the amount of crossing-over between the gene and the centromere with any high degree of accuracy, because two cytological variables are involved. However, we can obtain relative values, so that with chromosomes on which we have a large number of markers it should be possible to locate the centromere within rather close limits.

There is one difficulty which is apt to be encountered rather commonly in connection with interpretation of ratios. This is the problem of differential viability of various phenotypes. A great many recessive genes tend to reduce viability, and when a tetraploid is segregating for such a character, the introduction of another unknown variable renders the interpretation of results almost impossible. It is true that we can sometimes calculate the extent of differential viability in a diploid, and make corresponding allowances in calculating tetraploid expectancies. However, it is scarcely safe to assume that the extent of viability will be the same in the tetraploid as in the diploid. As a matter of fact, there is considerable evidence, especially in maize, that the deleterious genes accumulated in inbred diploid lines have a still more deleterious effect in the autotetraploid plants from these lines even though the change is one of quantity and not of proportion. Therefore, any results obtained from the study of tetraploid segregations that involve characters showing differential viability should be regarded as only very rough approximations.

Differential viability has an interesting effect on the values of *alpha* obtained from progenies of duplex and simplex hybrids. Since the proportion of recessives is usually decreased by differential viability, there is a tendency for the value of *alpha* to be decreased below what its value would be if the dominants and recessives were equally viable. However, the amount of decrease is not the same

for progenies of duplex hybrids as it is for progenies of simplex hybrids. For example, where double reduction is at a maximum, the value of *alpha* is 0.333, but differential viability eliminating 18.7% of the recessives is sufficient to change the value of *alpha* to zero in progenies from a simplex hybrid. On the other hand, in progenies from a duplex hybrid, the same amount of differential viability will reduce the value of *alpha* only to 0.208. In general, the same amount of differential viability will reduce the value of *alpha* in simplex progenies to a greater extent than it will in duplex progenies. Mather (59) noted just such differences in the progenies from duplex and simplex hybrids and postulated two different constants, *alpha* for simplex and triplex segregations, and *beta* for duplex segregations. He explained the difference on the grounds that changes of partner in pachytene pairing would affect the two types of segregations differently. The writer, in an unpublished report (52), suggested that this cytological explanation would not account for the difference between constants, and that differential viability appeared to be the most likely explanation. Recently, Mather (35) has acknowledged the incorrectness of his original explanation and suggested three other explanations, namely: chromosome differentiation, misclassification, and disturbed viability. We have already seen that chromosome differentiation tends to reduce the amount of segregation, and hence the value of *alpha*, in progenies from duplex hybrids, whereas it will have little or no effect on segregation in simplex progenies. Therefore, chromosome differentiation should produce an effect just the opposite of that which has been observed. It is also difficult to see how misclassification could explain the observed differences in *alpha* between simplex and duplex progenies. It appears likely that the most common source of error in classification would lie in the simplex plants of a progeny, since these are often similar to the nulliplex and might frequently be classified as such. Such an error in classification would increase the value of *alpha* above its true value, but the value of *alpha* from simplex progenies would be increased to a greater extent than the value from duplex progenies. Here again, the observed effect is the opposite from the one expected according to this explanation. As we have seen, differential viability would produce a result in accordance with observations, namely a value of *alpha* from simplex progenies less than that from duplex progenies. It is the only explanation yet proposed which readily accounts for the observed facts, and an explana-

tion which does not appear unreasonable, since we are dealing with a very common phenomenon.

In regard to the phenotypic results produced by genes, several unique phenomena are found in tetraploids. Whereas in diploids there are only three possible genotypes involving a pair of alleles, in tetraploids there are five. In two of these genotypes, the triplex and simplex, the proportion of dominant to recessive genes differs from the equal proportions always found in diploid hybrids. Often when two alleles are present in equal proportions, one is completely dominant over the other, but it may not be capable of completely masking the effect of three recessive genes in a simplex hybrid. For this reason, incomplete dominance is more common in tetraploids than in diploids. In *Primula,* for example, Sömme (77) has shown that in diploids the factor G for green stigma is completely dominant over the gene g for red stigma, affecting not only stigma color but also suppressing color in the flowers. In tetraploids, on the other hand, plants with the constitution $Gggg$ have green stigmas, but "the flowers have a darker shade, in some cases nearly as dark as that of the pure recessive form". In addition, the factor B for magenta flower color is completely dominant over red in the diploid, but in the tetraploid the class $Bbbb$ "exhibits colours varying from magenta to almost pure red". An even greater increase of incomplete dominance was found in connection with interactions involving the dominant white gene, W, and the green stigma factor, G. In tetraploids, the number of combinations of genotypes is so increased that they "may, according to their coloring, be arranged in an almost continuous series, ranging from pure white through lighter and darker shades to pure magenta or red". Sansome (73) has reported similar cases in tomato, where one dominant gene is not completely dominant over three recessives. In *Antirrhinum* (52) the basic color factor B and the magenta anthocyanin factor A appear to be completely dominant even in the simplex condition. However, the ivory flavone factor C will not completely suppress the formation of yellow flavone in simplex plants, so that $Cccc$ plants have considerably more yellow color than those containing two or more dominant C genes. It was also found that the basic anthocyanin factor F produced an almost continuous gradation of colors from full to very dilute as the number of recessive genes increased from zero to three.

One of the most striking genetic effects accompanying a change from diploidy to autotetraploidy is in connection with self-incompati-

bility. Lewis and Modlibowska (46, 47) have concluded that all natural autotetraploid forms of self-incompatible species are self-compatible. However, this conclusion is too broad, for there are some exceptions, such as the North Temperate autotetraploid species of *Tradescantia,* which are as self-incompatible as the diploids from which they arose. Some but not all of the experimentally produced autotetraploids of self-incompatible plants are self-compatible. The complex physiological mechanism, controlled by the *S* genes in diploids, is apparently upset in tetraploids so that the inhibition of pollen tube growth is in some way weakened. Where inhibition of pollen tube growth is rather weak in the diploid, it may disappear altogether in the tetraploid. Such seems to be the case in *Petunia,* as reported by Stout and Chandler (82), where the tetraploids were completely self-compatible. In *Oenothera organensis,* on the other hand, Lewis (46) has shown that inhibition of pollen tube growth is very strong in the diploid, and while it is reduced in the tetraploid, the reduction is not sufficient to allow fertilization to take place. In the cultivated apples, pears, and stone fruits, self-compatibility has been found much more common among polyploid forms than among the diploids (17, 18, 19, 47), but the genetic mechanism for incompatibility is so complex that a full explanation of the findings has not been possible. Howard (39) found that polyploid forms of *Brassica* and *Raphanus* are as self-incompatible as the diploids, but in this case, self-incompatibility is apparently not determined by oppositional alleles as in most self-incompatible plants.

Statistical Analysis of Tetraploid Segregation

The unfixed nature of tetraploid ratios introduces problems in statistical analysis not encountered in dealing with fixed diploid ratios. It is rather common in the literature for geneticists to refer to "chromosome segregation" and "chromatid segregation" as though these were two fixed ratios between which one must choose in fitting a set of data. As a matter of fact, these two types of segregation are, in reality, only ideal limiting ratios which are seldom attained, and probability favors the occurrence of ratios which are intermediate between these two limits.[1] The important statistical

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[1] As Mather (57) pointed out, random chromatid segregation is really not the upper limit as far as proportion of recessives is concerned. Data may simulate the random chromatid ratios due to the balance between 6/7 equational and 1/7 reductional separation. Complete equational separation at a locus will produce an even higher proportion of recessives.

problem is, therefore, not to determine which ratio the data fit more closely, but to find out to what extent the two opposing forces of reductional and equational separation have affected the data. Mather's index, alpha, conveniently characterizes a set of data in this regard.

It has been shown that the data on *Datura* published by Blakeslee, Belling, and Farnham (9) differed significantly from either the Müller or the Haldane ratios. On the other hand, it has been shown that Gregory's data (37) did not differ significantly from either type of ratio. These two examples bring out the point that large populations are necessary in order to draw valid conclusions in regard to tetraploid ratios. For example, it is necessary to have a population of at least 1,700 plants in order to be certain that any ratio obtained can be proven to deviate significantly from either a 35:1 or a 21:1 ratio.

The method of calculating expected ratios when the value of *alpha* is given is merely one of substituting this value in the formulae in Table I, column 5. The converse problem of calculating the value of *alpha* from observed data is discussed fully by Mather (57, 58, 59). Briefly the method consists of setting up a log likelihood equation in which the likelihood is expressed as the sum of the products of the observed number in each class and the log of the expectancy in terms of *alpha*. This expression is then differentiated with respect to *alpha*, and the derivative equated to zero. The solution of this equation is then the value of *alpha* giving the "maximum likelihood". The variance is then obtained by taking the second derivative of the original equation, substituting the value of *alpha*, and taking the negative reciprocal of the result. When data are available on several types of matings, the maximum likelihood equations will be rather complex and must be solved by successive approximations. When we have only data on progenies from selfed plants, as is often the case, the equations reduce to very simple forms. Thus, to calculate *alpha* from the progeny of a selfed simplex plant, we can use the equation: $alpha = 8\sqrt{z} - 4$, where z represents the percentage of recessives found in the population. The standard error will be $4\sqrt{(1-z)/n}$. For data from a selfed triplex plant, the value of *alpha* is $8\sqrt{z}$, and the standard error is the same as in the last case. The value of *alpha* derived from the progeny of a selfed duplex plant can be calculated from the formula: $6\sqrt{z} - 1$, and the standard error will be $3\sqrt{(1-z)/n}$.

Practical Significance of Tetraploid Segregation

Several of our horticultural crops are autotetraploid or behave partly as autotetraploids, and in recent years autotetraploids have been produced artificially among a wide variety of economic plants. For these reasons the problems of autotetraploid genetics are of considerable practical importance to the plant breeder. As an example let us consider the common problem in plant breeding of obtaining a true-breeding line. In diploids this is relatively simple, for when a selection is made in an F_2 or later generation which produces a uniform progeny, all plants in such a progeny are concluded to be homozygous, and one feels safe in bulking the seed and using it for an increase. Such procedure would be decidedly unsafe in an autotetraploid, for the selected plant may be triplex. In this case its progeny would be phenotypically uniform, but genotypically it would still be segregating, producing some duplex and even simplex plants which would segregate in the following generation. Just such a problem has been pointed out by Cadman (12) in connection with obtaining a strain of potatoes homozygous for resistance to virus.

Another problem which arises is the difficulty of obtaining multiple recessive types from a cross. As a simple example, suppose that a cross is made between two parents which differ by only three genetic factors, in an attempt to secure a plant recessive for all three. In a diploid the probability of obtaining such a triple recessive is 1 in 64. In an autotetraploid, however, the probability ranges from 1 in 8,000 to 1 in 46,656, depending on the amount of double reduction operating to increase the proportion of recessives. Obviously one must either grow very large populations or devise some method of obtaining the desired type in several steps.

Besides being of practical significance to the plant breeder, the study of autotetraploid genetics has another more indirect value, which consists of furnishing an approach to the study of quantitative inheritance. This problem has been one of the most baffling and complex encountered in the field of genetics, and is still far from being solved. Yet it is of the utmost economic importance, since a great majority of the genes which contribute to the value of economic plants are quantitative in character, and a thorough understanding of their hereditary behavior is greatly to be desired. Lindstrom (50) states that "comparison of quantitative inheritance in $2n$ and

$4n$ affords another approach to the problem of quantitative inheritance", and he has taken the first step in this direction through a study of quantitative genes in diploid and tetraploid tomatoes. With the increase of tetraploid plants available for study, further work along this line should be possible and should prove highly profitable.

SUMMARY

Three main theories of autotetraploid segregation have been propounded, the Muller hypothesis based on the random assortment of chromosomes at meiosis, the Haldane hypothesis based on the random assortment of chromatids, and the Mather hypothesis in which the ratios are considered to be not fixed but varying according to the amount of quadrivalent formation and the distance of a gene from the centromere. The first two hypotheses are special cases of the last, which is, in general, the most satisfactory for explaining observed data.

While many autotetraploids do not lend themselves readily to genetic analysis, due to sterility, chromosome differentiation or lack of good genetic characters, fairly extensive studies have been carried out on the autotetraploid genetics of ten species in as many genera.

The cytological variables that affect autotetraploid segregation are mode of pairing, formation of quadrivalents, and number and position of chiasmata. In progenies unaffected by variables other than these, it is possible to gain an estimate of the position of the gene involved with respect to the centromere.

Differential viability markedly affects some of the autotetraploid ratios, and can account for some of the discrepancies between observed data and expectancies.

Incomplete dominance is a more common phenomenon among tetraploids than among diploids, due to the greater number of genotypes possible for any given pair of factors.

On account of striking differences between the genetics of diploids and tetraploids, plant breeding procedures applicable to diploids must frequently be modified in dealing with tetraploids.

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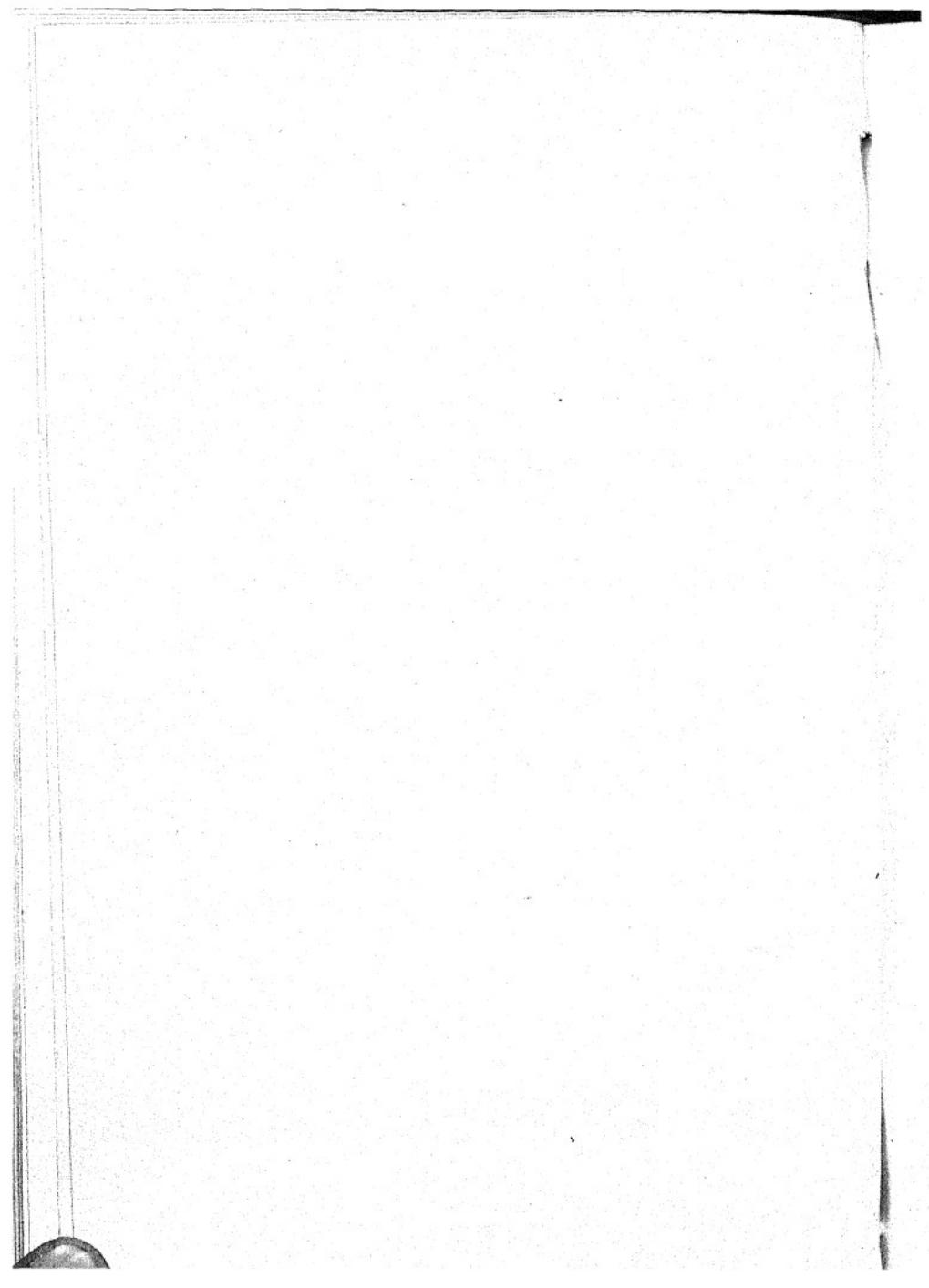
A CRITICAL SURVEY OF THE PRESENT STATUS
OF PLANT EMBRYOLOGY

DONALD A. JOHANSEN

INTRODUCTION

At the outset of this article it might be stated that the most that can be said for the present status of plant embryology is that it is in a curious muddle. The main reason underlying this state of affairs is that very few botanists seem to realize that plant embryology has become an exact science. There are actually people who, in effect, deny that there is anything to this division of the botanical sciences. For instance, not long ago a botanist in India, while discussing certain species belonging to the Scrophulariaceae, made the following incredible statement: "A careful study of the development of the embryo shows that the stages are similar to those of a typical dicot embryo, and it is superfluous to describe these stages elaborately".

It is one of the commonest but most completely erroneous beliefs among botanists generally that there is such a thing as a "typical dicot embryo". Many authors dispose of the question of the embryogeny of the plants they investigate by stating that "it follows the normal Angiosperm (or dicotyledonous) type of development", blissfully ignoring the fact that no such development has ever been described. It is incomprehensible how such a "normal" method could be imagined to prevail for all angiosperms, or for either the dicotyledons or monocotyledons. This is but another example of the widespread misuse of the words "normal" and "typical" in plant morphology; sight seems to have been lost of the fact that what may be typical for one group of plants in some one respect is precisely as abnormal or atypical for other groups in the identical respect. We may recall that the so-called "normal type" of megagametogeny is common enough and may be "normal" for those plants in which it occurs, but it is quite abnormal for plants whose megagametogeny conforms to, say, the *Fritillaria* Type or

the *Oenothera* Type.   Each of these types is in every sense "normal" for the plants in which it prevails; consequently, the only possible conclusion is that there is no such thing as a "normal type" for either megagametogeny or embryogeny.

One purpose of this article is to dispel the fatuous belief described above and to demonstrate, on the contrary, that it is far from being a superfluous matter to investigate the embryogeny of a particular plant carefully, cell by cell from the zygote to at least the later stages of proembryonic growth, and to publish such accounts, if only for the reason that the total number of angiospermic species which have been thoroughly studied embryologically amount to scarcely a hundred out of the many thousands of species which have been described.

The present account is concerned solely with angiosperms. Others are perhaps better qualified to discuss the gymnosperms; in any event, the embryology of this latter great group has been established on a more solid basis, thanks principally to the magnificent researches of Buchholz and his students, although the great mass of available information still remains to be compared and integrated as a whole.   It should be mentioned in passing that this article is the outgrowth of the writer's activities during the preparation of a text on plant embryology (1); the opportunity is thus afforded to discuss a number of matters which could not be included in that book.

No distinction is being made between dicotyledons and monocotyledons, for the most part.   It will perhaps be as much a surprise to many readers, as it was at first to the writer, to learn that there is absolutely no fundamental difference between these two taxonomic groups in so far as the ontogeny of the proembryo is concerned.

WHAT IS PLANT EMBRYOLOGY?

That would seem at first glance to be a stupid question, but as a matter of fact it is difficult to determine what many people understand by the term "embryology".   Its meaning seems to vary according to the individual author.   Many Scandinavian and German workers clearly apply it to embrace megasporogeny and megagametogeny alone with complete elimination of embryogeny.   That is, they follow out all the phases from development of the ovule to maturation of the megagametophyte, and there stop.   In a way,

since the series of events culminating in the formation of the egg are the precursors of the second series of events which end with the mature embryo, this might be described as embryology in an excessively broad sense.   However, as a practical matter, it is confusing and deceptive; moreover, it is very annoying to the "pure" embryologist who is put to much trouble to procure obscure journals only to find that a title in which the word embryo is used in some connotation turns out to be misleading in that the embryogeny is not dealt with in any way whatever.   There are literally hundreds of papers of this sort, all parading under false pretenses. Development of the ovule, strictly speaking, comes under the heading of anatomy.   When megasporogenesis and megagametogenesis are separate processes, the first should be described as megasporogeny and the second as megagametogeny; when the two are consolidated into one process, it is simply megagametogeny.   Embryogeny does not come into being until syngamy occurs; that phenomenon marks the commencement of the new sporophytic generation. Those who use the term embryology to cover all these processes overlook the fact that they are dealing with two separate generations, and with two aspects of one of these.   Megasporogeny and megagametogeny in other than certain apomictic plants relate to the gametophytic (reduced or haploid) generation; embryogeny to the sporophytic (unreduced or diploid) generation, save where haploparthenogenesis is concerned.   Again, development of the ovule concerns the preceding sporophytic generation.

   All matters considered, it is far more preferable to consider embryology in the strict sense alone; that is to say, as it relates solely to the ontogeny of the embryo itself.   Adherents of this conception may be described as belonging to the Souèges School; those who prefer the extremely broad conception described in the preceding paragraph might be designated as conforming to the Mauritzon School.   To the Souèges School development of the embryo is the all-important matter; to the Mauritzon School, which has a decidedly sciolistic conception of embryogeny, development of the embryo as such is a relatively insignificant consideration.   Each School doubtless has its place in the botanical scheme of things, but when one gets down to cases, it must be said that the Mauritzon School rarely makes an actual contribution to our knowledge of the precise embryogeny of the species which are discussed.

DEFINITION OF PLANT EMBRYOLOGY

As was indicated previously, plant embryology has advanced to the point where it is now one of the most exact of the botanical sciences. Few persons seem to realize, or to appreciate the significance of, this fact; the writer must confess that he certainly did not comprehend the extent to which our precise knowledge of embryology had grown until after a laborious digestion of Souèges' extensive writings. No one has hitherto called specific attention to the basic contributions of this French investigator. (A detailed discussion of Souèges and his contributions will follow.)

Plant embryology may be divided into two main divisions: (*a*) General Embryology and (*b*) Special and Comparative Embryology (1, 3).

The first division, General Embryology, may be subdivided into three groups of more or less related topics:

1. Embryonic Morphology. This embraces the static phases of embryology, and these in turn depend upon whether the external aspects alone are considered, in which case it may be described as Embryography, or whether specific internal aspects are under consideration, in which instance either Cytology, Histology or Anatomy is the term to be employed as the occasion requires.

2. Embryonic Physiology. This embraces the dynamic aspects of embryology. These aspects are very rarely dealt with by embryologists and are generally left to plant physiologists. One example is the cultivation *in vitro* of more or less immature embryos removed from seeds.

3. Embryogeny. These phases, with which the great majority of embryologists are concerned, embrace the kinematic aspects of embryology. They may be separated into individual phases, with appropriate designations, as follows:

a. Embryogenesis proper: origin of the embryo.

b. Embryotectonics: architecture of the embryo.

c. Embryogenergy: destination or function of the embryo.

d. Embryonomy: exposition of the laws of embryonic development.

Special and Comparative Embryology require no subdivisions, since these topics relate to individual species, to well defined genera, occasionally even to entire families, and therefore follow phylogenetic lines more or less. There is a certain degree of over-

lapping, however, since species in very diverse and totally unrelated taxonomic families may conform to the identical embryonomy.

CONTRIBUTIONS OF SOUÈGES TO PLANT EMBRYOLOGY

One of the most inexplicable features of plant embryology of the present time is the extent to which the classical and fundamental researches of the French botanist, Souèges, are ignored. In fact, the general disregard of his work can be described only as enthusiastically unanimous.

Why this should be is difficult to comprehend. The writer has record of several papers on species which had been already fully investigated by Souèges, but in which no mention whatever of the earlier work is made.

A few biographical details regarding Souèges, since he appears to be comparatively unknown to American botanists, may not be amiss. Certain authors have given his initials as "M. R.," apparently in ignorance of the fact that the "M." is merely the abbreviation for Monsieur. His full name is Etienne Charles Réne Souèges. He was born in 1876. His academic training was not solely that of a botanist, as he has received French university certificates in mineralogy, biological chemistry, zoology and pharmacy as well as in botany; he acquired the doctorate from the University of Paris in the natural sciences in 1907. He has held no position corresponding to that of a professor in an American university; since 1912 he has been "Pharmacien en chef des Asiles de la Seine". The presumption is that his position is (or at least was, before the war) analogous to that of the head of a department of pharmacology in a medical school, but he, unlike so many American pharmacologists who make an avocation of the microscopic anatomy of the plant components of the materia medica, has made an avocation of plant embryology. In his later years, Souèges acquired a consuming ambition for election to the French Académie des Sciences, which had a certain deleterious effect on the mode of presentation of his investigations, particularly in the Comptes Rendus de l'Académie des Sciences. This ambition culminated in the appearance of his "Titres et Travaux Scientifiques" in 1934 (2); this, in brief, is an account of the personal scientific history and accomplishments which candidates for election to the Academy are required to present. The preparation and publication of such

a document may appear highly egoistical to an American; but that is merely the French way of doing things. The total number of titles of Souèges' published embryological papers exceeds a hundred; in addition, there are about ten brief books or pamphlets on such subjects as the history of plant embryology, the laws of embryogeny, and microtechnique as applied to embryology.

Souèges has placed descriptive angiospermic embryology on a high level of precision; it is so elevated, in fact, that only a remarkably few other workers, notably Bhaduri and Noll, have been able to comprehend, to criticize intelligently, and to duplicate with reasonably close imitation, his unique methods of descriptive embryology. Souèges has been a careful, painstaking and methodical worker; he has stated on several occasions that it required years of effort to work out the complete embryonomy of many of the species with which he dealt.

Souèges himself is not without his shortcomings. He is prone to direct little or no attention to the work of others in his brief articles which have appeared in the Comptes Rendus, although he does deal fully and completely with the literature in his more extended papers, particularly those which have appeared in the Bulletin de la Société Botanique de France. The first journal apparently places excessive emphasis on brevity. His descriptions of embryonomy in the Comptes Rendus are models of preciseness and completeness, but are not always strictly accurate from other standpoints. His use of the term "cellule apicale" for the outer cell of the two-celled proembryo should particularly be condemned; the writer might be in error in making this criticism if the term "apicale" has some meaning other than that of "apical" to a Frenchman. In any event, this cell, as has been recognized by several other workers, is not an apical cell in any sense of that term; it should be designated as the terminal cell. Souèges uses the term "dermatogen", in common with many another botanist, to apply indiscriminately to isolated epidermal initial cells, wherever these may be formed on the developing embryo, and has nowhere shown any indication of realizing that this word can apply only to the derivatives of a definite group of dermatogen initials located at the apex of the root proper, or from a group of dermatocalyptrogen initials. The epidermis of the embryo proper is never developed by such initials, but the epidermal initials arise independently in various portions, either

simultaneously or oftener at different developmental stages. The
writer on occasion was unable to understand precisely what Souèges'
conception of the root cortex included: at times he appeared to
refer only to the cortex proper; in other instances he appeared to
include all structures composing the partially differentiated embry-
onic root. This, obviously, is confusing. It would seem that
Souèges is simultaneously at his best and his weakest when
describing the origin and development of the primary root in the
embryo. That is to say, he follows out the sequence of divisions in
the radicular region with great precision, but is careless in his use
of terms to describe the various component portions.

Despite all his personal idiosyncrasies, Souèges and his work
on plant embryology can no longer be ignored by future workers
in that field. Even if one fails despite strenuous effort to attain
his level of clear description of embryonomic processes, the attempt
should be made to do so. It should also be suggested that his mode
of description of the various embryonal regions be retained; the few
attempts that have been made to change his established zones of
embryonal growth have resulted only in great confusion.

For the purpose of describing accurately the sequence of cell
formation in the proembryo and embryo, Souèges has made it clear
that these structures should be observed, described and figured with
the terminal cell of the two-celled proembryo (or the embryo proper
of the mature structure) pointed up or away from the observer,
and with the basal cell (or the radicular end of the mature embryo)
directed down or toward the observer. Numerous exponents of
the Mauritzon School apparently never could make up their minds
as to which way the embryo should be arranged; generally they
depict it in exactly the same position that the mature megagameto-
phyte is customarily figured—that is, with the micropylar end upper-
most and the chalazal end lowest—or the reverse of the Souèges
manner. Essentially, this matter should be one of no particular
significance, since the megagametophyte and embryo in nature
may both be oriented in any conceivable position, but it makes for
greater stability and precision in description if the embryo be always
considered from the one and the same position.

THE LAWS OF EMBRYOGENY

From a consideration of the great regularity present during the
development of the proembryo of a given species, Souèges was led

to the formulation of a number of laws, each of which is truly specific when applied to that species. Those which succeed these fundamental characteristics during the ontogenesis of the embryo and which prevail in the development of definite tissues derived from the proembryonic initials or are already roughly delimited in the proembryo are not specific but are merely characteristic of the tissues in those individual species whose construction they govern. It is on this basis that the monocotyledons cannot be separated from the dicotyledons embryologically; during the second cell generation, the proembryo of the monocotyledonous *Lilium parryi,* to take one instance, divides precisely as does that of the dicotyledonous *Godetia amoena,* so that the two genera conform to exactly the same fundamental embryonomic type. It is not until much later that the secondary cotyledonary characters arise; such characters are not of basic importance.

The various laws may now be defined briefly.

(*a*). Law of Parsimony.—The pertinency of this law has apparently not occurred to Souèges, but it is most decidedly applicable to the field of plant embryology. In fact, it may be the fundamental law and all other laws may be derived from it.

As originally propounded by William of Occam (ca. 1270 to ca. 1350),[1] and known as "Occam's Razor", the law stated that "Entia non sunt multiplicandi praeter necessitatem". Later the same principle was discussed at greater length by Sir William Hamilton, a Scottish philosopher (1788–1856), and was called by him the "Law of Parsimony".

A very literal translation of the "Razor" is: "Entities are not to be multiplied beyond necessity". However, freer translations are possible and have been made. In so far as its application to plant embryology is concerned, this law may be stated thus: No more cells are produced by an embryo than are absolutely necessary.

The implications of this statement are obvious to any person who has ever carefully followed the development of the proembryo of almost any plant. Each and every cell has a reason for its existence; its origin can be demonstrated; its destination can be determined; and its position is invariably the same. A superfluous cell would seriously upset the harmonious balance of developmental

[1] The writer is indebted to Dr. Philip R. White for the information contained in this paragraph. See also: J. P. Givler, Occam's Razor and Mendel's peas. Jour. Elisha Mitchell Sci. Soc. 48: 108–112. 1932.

factors present in the proembryo, and would undoubtedly lead to the death of the latter.

The one flaw in the rigid application of the law is that we do not yet know what is the real role or function of the suspensor, particularly in those Types or Variations in which the derivatives of the basal cell, which usually gives rise to the suspensor, contribute nothing to the construction of the embryo proper. In such instances the apparently superfluous cells of the suspensor may be disregarded.

(*b*). Law of Origin.—In any particular species, the sequences of cell formation may be established in such a manner that the origin of the cells may be defined in exact terms by referring to the one or to the other of the terms of the sequence.

It is hardly necessary to state that embryonic cells arise solely by simple successive bipartitionings of the zygote and its derivative cells.

The law of origin is the second most important of the embryogenic laws, and the principal branch of embryogeny, embryogenesis, rests on this law.

(*c*). Law of Numbers.—The number of cells produced by different cell generations varies with the species and depends upon the rapidity of segmentation in the cells of the same cell generation.

The rapidity of segmentation of a cell is calculated in determining the number of elements which the cell engenders in terms of successive cell generations. The law of numbers is identical with the law of rapidity and becomes evident in fundamentally different characters, as do the other embryogenic laws. Among other things it permits the establishment of particularly instructive resemblances between taxonomically diverse species.

The terminal cell of the two-celled proembryo generally segments more rapidly than does the basal cell and it usually gives rise to all of the embryo proper. The basal cell ordinarily contributes no more than the suspensor and portions of the root apex, and divisions occur less rapidly. There are instances, however, in which the basal cell segments more rapidly to form an extensive structure, and it may also contribute a greater portion of the embryo proper (the suspensor is considered not to be a portion of the embryo proper).

(*d*). Law of Disposition.—In the course of normal embryonic development, the cells are constituted by divisions in clearly deter-

mined directions and appear to occupy positions in accordance with the role which they must play in the future development of the embryo.

The laws thus far mentioned concern the relation of cells *to time,* while the law of disposition defines their relations *to space.*

All the dispositions which the cells of an embryo might assume are governed, in a fundamental manner, by the axial symmetry generally presented by both the proembryo and the embryo. This axis is identical with the axis of growth of the plant subsequent to germination.

The positional direction of the walls which segment a cell may be defined by taking account of their axial symmetry and by referring to the geometric figures which obey this symmetry. Cells separated by walls are thus similar to geometric solids: they possess the same faces, edges and angles, which allows the precise statement of their forms and the comprehension of their mode of assemblage. This branch of embryology which seeks to establish the laws governing the grouping of cells, the relations which they offer to each other in space, and which are applied to describe all the architectural dispositions of the embryonic body, constitutes embryotectonics.

(*e*). Law of Destination.—The cells of the proembryo of a given species, when development is normal, give rise to clearly determined parts, and always to the same parts, of the embryonic body.

This law appears to admit, in the fashion of a postulate, the specificity of the cell, besides which it affirms that each cell has a predetermined rôle to play. Among plants, the destinations of cells are not rigidly predetermined, since in addition to their actual potentialities the cells conserve a more or less considerable total of virtual potentialities which permits them to produce other parts of the embryonic body in case ordinary conditions of development become modified.

The law of destination is closely related to that of disposition. The first states, in effect, that the embryo and the plant represent a vertical edifice and that the fundamental regions of the body are situated in this edifice at different levels corresponding to the uni- or pluricellular embryonic stages. The law of disposition determines the mode of separation and the internal composition of the edifice.

The law of destination reveals the truly essential differences in embryogenic characters, in that it permits the establishment of the constant connections which, directly compared, constitute a source of precise indications of specific relationships between different plants. Determination of these relationships—in effect, the nature of the work performed by the embryo, the constructive rôle of its parts, and in sum the destination of each cell of the proembryo—is designated by the name of "embryogenergy".

CLASSIFICATION OF ANGIOSPERM EMBRYONIC TYPES

Up to the present time no one has attempted to formulate a definite scheme including all embryonomic types and variations so distinguished as to be readily recognizable. Souèges, in one or two of his later papers, mentions "Megaarchtypes", but the writer has been unable to find a scheme employing such terms in any of this investigator's publications that could be procured. Unsuccessful attempts were made to communicate with Souèges, both directly and via Swiss intermediaries, in an effort to ascertain whether he had actually prepared a classificatory scheme. Apparently he has done no more than to express the hope that a precise classification could some day be prepared.

The beginning, therefore, is the one made by Schnarf in his "Embryologie der Angiospermen" (4). He took the name of the family to which belonged the species whose embryonomy was most completely known, and designated it as the Type. It is clear that Schnarf was motivated by the fundamental laws laid down by Souèges, although he does not mention the fact, in his selection of the Types. Thus, he employed the terms "Cruciferen-Typus", "Astereen-Typus", Solanaceen-Typus", "Chenopodiaceen-Typus" and "Caryophyllaceen-Typus". Use of the designation "Type" appears to have become generally accepted, and the precedent established by Schnarf is fully worthy of perpetuation, since there is no acceptable alternative. However, the use of purely German names is open to objection; it seems to be better to take the root of the family name rather than the latter itself and to attach to this root the Latin suffix -ad. Schnarf's designations, with one exception, are retained in the revised form. His use of the Cruciferae (Brassicaceae) must be abandoned in favor of the Onagraceae, since species belonging to the latter family display a more funda-

mental type of embryonomy than does *Capsella bursa-pastoris,* the
basis of the "Cruciferen-Typus".

The Types are based upon the operation of each and every one of
the laws of embryogeny described above. Specifically, separation
of one Type from another depends primarily on the plane of the first
division in the zygote (whether longitudinal or transverse), secon-
darily, on the behavior of the terminal cell of the two-celled pro-
embryo, when the plane of the first zygotic division is transverse,
during the first and subsequent cell generations, and less directly
upon whether the basal cell contributes something or nothing to the
construction of the embryo proper.

The six Types which are now recognized may be best described
briefly by means of a key:

I. The zygote divides by a purely or essentially longitudinal wall.
                                                                Piperad Type
II. The zygote divides by a transverse wall.
   A. The terminal cell divides by a longitudinal wall during the second cell
      generation.
      1. The basal cell has no or only a minor part in the construction of
          the embryo proper .... Onagrad Type
      2. Both basal and terminal cells contribute, more or less equally,
          to the construction of the embryo proper ......Asterad Type
   B. The terminal cell divides by a transverse wall during the second cell
      generation.
      1. The basal cell has no essential part in the construction of the
          embryo proper.
          a. The basal cell undergoes no further division and becomes
             a large suspensor cell (when a suspensor of two or more
             cells is formed, these are always derived from the termi-
             nal cell) .... Caryophyllad Type
          b. The basal cell usually forms a suspensor of two or more
             cells .... Solanad Type
      2. The basal cell contributes more or less to the construction of the
          embryo proper .... Chenopodiad Type

No good purpose would be served by a fuller discussion of these
types in the present connection, since they are described at con-
siderable length elsewhere (1).

Under each Type there are from two to numerous subdivisions,
styled Variations. Variations have never been specifically des-
ignated as such; they are being recognized for the first time in the
writer's text. A Type is fundamental; a Variation is based upon
modifications of the basic type. These modifications are more or
less precocious differentiations, differences in the number of cells
which participate in the construction of the suspensor, in the form
or very variable dimensions of this structure, or even in its absence,

or differences in the rapidity of the segmentations which may occur earlier or later in favor of one of the cells produced by the same division. Variations are most conveniently designated by the name of the genus whose embryonomy departs in a minor fashion from that of the Type to which it is attached.

While it is a fairly safe assumption that the Types will stand the test of time, Variations are another matter. Not all are as clearly definable as could be desired. It was also recognized that as future research reveals the necessity, it is inevitable that new Variations will need to be erected under the appropriate Types.

The number of Variations is greatest under the Onagrad Type. Dicotyledonous genera so assigned include the following: *Capsella, Alyssum, Veronica, Catalpa, Myosurus* (including all Ranunculaceae), *Lythrum, Euphorbia, Mentha, Ruta, Trifolium* and *Lotus;* while the monocotyledons represented include *Anthericum, Lilium, Heloniopsis* and *Juncus.* Anyone familiar with the taxonomy of the angiosperms will recognize that these represent the most diverse families, yet all conform to fundamentally the same embryonomic Type and differ only in minor, secondary respects.

EMBRYOLOGICAL DISTINCTIONS BETWEEN DICOTYLEDONS AND MONOCOTYLEDONS

Some readers may question the statement previously made that there is no fundamental difference between the two great classical taxonomic groups among the angiosperms. The complete lack of recognizable basic differences was first enunciated by Souèges, whose reasoning leaves little or nothing for debate.

That there might be a difference arises from the fact that many authors have advanced the speculation that monocotyledony arose from dicotyledony through abortion of one cotyledon. This has never been proved as an indisputable fact, and can be shown by the embryogeny of almost any monocotyledonous plant to be palpably false. In effect, the two points of growth in the cotyledonary zone which occur among most dicotyledonous plants, in symmetrical position with reference to the embryonic axis, do not possess their homologues among monocotyledonous plants at any moment in the ontogeny of the embryo. The point of growth of the single cotyledon of monocotyledonous plants is located precisely on the embryonic axis, and no other symmetric point of growth exists.

Consequently, there can not be abortion of a second point of growth, since such a second point has never been found to exist at any time.

It is true, of course, that certain dicotyledonous plants have been described as aborting one cotyledon, thus attaining a pseudo-monocotyledonous condition. The one cotyledon which develops to maturity in such plants is always lateral with reference to the embryonic axis and is never truly terminal as in typical mono-cotyledons. Vestiges of the aborted cotyledon can usually be detected, generally at an early stage in the development of the embryo.

The embryological evidence, incidentally, would seem to furnish excellent arguments, if not conclusive ones, for those who favor complete abandonment of the separation of angiosperms into two groups.

APOMICTIC EMBRYOS

In the realm of apomictic phenomena conditions are extremely unsatisfactory. Various attempts have been made to straighten out the confusion, but from the embryological standpoint none of these endeavors has entirely succeeded in placing the subject on a sound basis. Some geneticists, in fact, have gone off on a wide tangent in an attempt to explain a purely embryological phenomenon on a basis of genetical terminology. It appears to be necessary to point out the fact that the mere presence of supernumerary mega-sporocytes, quartets, functioning megaspores or megagametophytes (whether normally or irregularly constructed) is in no sense proof that the plant concerned is regularly or sporadically apomictic. Such extra structures are far more prevalent than appears to be realized, and it is generally a matter of no particular significance. They are, for example, very common in *Fuchsia, Godetia, Lilium, Crepis, Nicotiana* and numerous other genera, but eventually dis-integrate during later growth stages and never produce apomictic embryos. Indubitable proof of the presence of an embryo or two to several embryos in such supernumerary ovular structures must be presented before the plant in which they are found can definitely be considered as a regular or sporadic apomict. The majority of papers on apomictic phenomena have not presented such proof and consequently are of no value from the embryological standpoint.

POLYEMBRYONY

The situation with respect to polyembryony is equally unsatis-

factory. Buchholz has all but completely eradicated the confusion regarding it in the conifers, but no one has attempted to do the same for the angiosperms.

Matters depend considerably on the definition of polyembryony. Most authors apparently would define angiospermic polyembryony as the existence within the same ovule of two or more embryos, regardless of their origin. It is in precisely this respect that confusion lies. That is to say, no attempt is usually made to distinguish the actual origin of the various embryos that might occur. In most of the so-called instances of polyembryony, the supernumerary embryos are derived from the nucellus. The phenomenon has generally been styled "nucellar embryony", but the proper designation is "adventitious embryony" (to include instances of embryos arising from the inner integument), and such embryos must be classified as apomictic.

To cite another instance, if one of twin seedlings is haploid and the other diploid, it can ordinarily be taken for granted that the diploid seedling arose from a fertilized egg, while the haploid seedling developed either from an unfertilized synergid or from the nucellus or inner integument. Such an example should not be classified as polyembryony but as normal embryogeny accompanied by apogamety or adventitious embryogeny.

If we take the cue for the definition of true polyembryony from similar circumstances in conifers, there are only two causes of true polyembryony among angiosperms:

(a). A normally fertilized egg gives rise to a more or less multicellular body from which several potential embryos may arise. These extra embryos originate either from a massive suspensor produced by the basal cell of the two-celled proembryo, as in *Zauschneria latifolia* and certain orchids, from an embryonal mass produced mainly by the same cell, as in *Tulipa* and *Erythronium*, or by presumable budding from epidermal cells of the embryo proper, as in *Nicotiana rustica*. In most instances only one of the several embryos actually attains maturity.

(b). A filamentous proembryo first develops from the zygote, then its terminal cell splits longitudinally into two embryonal initials. Until very recently the evidence in all recorded cases was circumstantial, but the phenomenon has been authentically described in the orchid *Cymbidium bicolor*.

EXTENT OF EMBRYOLOGICAL STUDIES

A vast amount of careful work still remains to be done before the subject of plant embryology could be said to begin to show signs of exhaustion. The preponderant majority of angiospermic families have never been investigated with the slightest degree of completeness; in fact, there are many entire orders in which no species has ever been studied, save perhaps in a very superficial manner. These orders include the following: Anonales, Loasales, Podostemonales, Dilleniales, Coriariales, Pittosporales, Passiflorales, Theales, Tiliales, Malpighiales, Cunoniales, Garryales, Myricales, Balanopsidales, Casuarinales, Olacales, Meliales, Alstroemeriales, Dioscoreales, Agavales, Palmales, Cyclanthales and Haemodorales. Under still other orders a few families have been studied more or less, but the remaining families are still unknown embryologically. Under the Leguminosales, for instance, there are many investigations on both the Papilionaceae and Mimosaceae, but nothing whatever is known for the Caesalpiniaceae. Of course, it is recognized that many families, and even some orders, represent species which grow only in tropical or out-of-the-way localities; but nevertheless, many occur within easy access of properly equipped investigators in the North Temperate Zone.

THE HANSTEIN HISTOGEN CONCEPT

The original concept of the nature of histogens as devised by Hanstein, added to by Janczewski, and elaborated by Haberlandt, is plainly one that was presented far in advance of its time and thereby has suffered considerably. The bulk of the criticism has justly been directed against application of the theory to stem apices, far less to its application to root tips.

The only purpose of the writer in bringing up the subject is to point out one fact that seems to have been completely overlooked both by the originators and by later critics. It is fully conceded that the critics are on solid ground as far as their criticisms go; the point is that in no case has any one covered a particular phase completely. All writers have dealt with fully mature structures, whether they be stem apices or root tips, and in the case of the latter it has never been any too clear whether primary or adventitious roots were considered. No one seems to have begun with the actual origin of the histogens in the young embryo and worked

from that starting point up to maturation of the histogens in the seedling or adult plant.

It is the writer's strong contention that the start must be made in the young embryo—at the very first histogenic cell. Otherwise, only an isolated phase of the entire story is told. It is for this reason that it is believed that the histogen concept was prematurely presented; it was formulated at a time when nothing was understood of the laws of embryogeny which underlie the origin and development of histogens in a most fundamental manner.

It is impossible to comprehend how histogens arise, what their nature is, how the component cells are interrelated, and so on, unless the start is made in the embryo. The origin and development of the tissues of root tip and root cap can not be understood from a study of sections of adult tips. The same statement applies to the stem apex. Again, it needs to be borne in mind that the primary root has an origin entirely different from that of adventitious (lateral) roots. Structurally, the two may superficially appear to be identical, but that they actually can be so despite their totally different modes of origin has never been demonstrated.

The histogen concept needs to be restated anew from an entirely different standpoint, that of the embryonic origin of the histogens. It is entirely probable that the types of histogens found in a particular plant are intimately correlated, if not identical, with the Type and Variation to which that plant conforms embryonomically.

EMBRYOLOGY AND GENETICS

There undoubtedly exists a definite relationship between embryology and genetics. The latter term is here understood to refer mainly to crossing and hybridization experiments. Here the connection is clear-cut; in other aspects the interrelationships are still obscure.

A number of tentative embryological postulates relating to the crossing of closely or distantly related species can be laid down as a result of reflection on known embryological processes and known genetical phenomena. No attempt to prove these postulates is being made here; their presumable existence can only be suggested, and it remains for future investigations to demonstrate their validity or lack of such.

It is a well known genetical fact that zygotes resulting from

interspecific crosses divide once, then perish without growing beyond the two-celled proembryonic stage. What causes this arrested development? An investigation of the embryogeny of each of the parents should provide the clue. It is entirely possible that one parent may conform to some one set of embryonomic laws and the other to a different set of laws. Taking it for granted that conformation to a given set of embryonomic laws is an inherited character which does not permit of modifications in the same sense that differences in chromosome numbers can be reconciled, it follows that the hybrid zygote has received two conflicting sets of embryonomic characters in such instances where the respective parents conform to opposing embryonomic laws. Since the transverse division of the zygote is all but universal, the first division of this cell presents no difficulties, but these do arise when the time for the second division arrives. The incompatibilities between the inherited sets of embryonomic laws can not be reconciled, and death therefore ensues. To take a suppositious instance, let us assume that one parent conforms to, say, the Onagrad Type, in which the first division in the terminal cell of the two-celled proembryo is longitudinal, and the other to, say, the Caryophyllad Type, in which that division is transverse. What can the hybrid proembryo between two parents with such directly opposite characters do when the time for the terminal cell to divide comes? It can not divide in two opposite planes simultaneously. There is, in effect, nothing that it can do; therefore, it does nothing and because of the lack of further growth the proembryo perishes. It may therefore be postulated that the zygote of a hybrid between two parents with opposing embryonomic laws inevitably perishes. Furthermore, following the same line of reasoning, it may also be postulated that only those crosses are successful wherein both parents conform to the identical embryonomic laws.

In the preceding discussion, conformation to embryonomic Types was considered. There are numerous other recorded instances in which hybrid embryos developed into few- to several-celled monstrosities. These require an explanation somewhat different from the one given above. In such cases it may be presumed that each parent conformed to a different Variation under the same Type. Incompatibilities between Variations are not so difficult to reconcile as are those between Types, but it is still not always possible for the proembryo to overcome the differences completely. Ir-

regularities in construction of the embryo, generally manifested in the form of short-lived monstrosities, are all but inevitable in such cases. From this it may be postulated that in such instances where the parents of a hybrid conform to different Variations under a given Type, irregularities in the construction of the hybrid embryo are inevitable and generally end in the death of the latter.

It has now become quite clear that the basic reason why it is so easy to make interspecific and intergeneric crosses in the Onagraceae, despite other genetical or chromosomal differences between the parents, is that the entire family constitutes a remarkably homogeneous embryonomic unit. Differences in cytological or other genetical details clearly are not of as fundamental importance as is adherence to the identical cell-by-cell development of the proembryo and embryo. There are species which display divergences from the fundamental Type, particularly in the genera *Epilobium* and *Zauschneria*. It is demonstrable that when a form which either constantly or sporadically exhibits embryonal abnormalities is used as one parent in genetical experiments, the differences which this parent displays are reflected, usually in an exaggerated manner, in the progeny. At least there is no recorded instance in which the irregularities of one parent are overcome or erased in the offspring. All records reveal continued irregularities; the crosses frequently do not result in viable seed, and the cause, at least in those instances where sufficient details of the embryogeny were presented, is clearly due to abnormal development of the proembryo and its early demise.

It is apparent that future genetical investigations involving crossings between species and within the same species will be immeasurably enhanced in both theoretical and practical value if the embryogeny of both parents and offspring is taken into account along with cyto-genetical details. Those factors so far considered by geneticists in explaining their results are inadequate; the embryonomy needs to be evaluated along with the cytological and other genetical factors. It may, at least in very many cases, prove to be the deciding factor in determining the success or failure of hybridization experiments.

MICROTECHNICAL ASPECTS OF EMBRYOLOGY

It is admittedly a slow, tedious and tiresome job to prepare material of the majority of plants for embryological study. Not

many modern investigators seem to be possessed of the patience and perseverance of a Treub or Strasburger of the older school of botanists or of a Souèges or Buchholz of the present time to make thoroughgoing embryological investigations. Being an expert microtechnician is not alone sufficient; there is a good deal more to it. Strasburger, for instance, was one of the earliest microtechnicians, doing much of his sectioning with a hand razor, and his embedding and staining methods must have been crude in comparison with the modern procedures now available, yet his descriptions and drawings remain superior to many modern counterparts.

One needs plenty of patience and endurance to dissect out literally thousands of ovules which are tiny and difficult to manipulate in most plants, to get such quantities of material embedded, the skill to microtome the ovules in precisely the right plane to secure median longitudinal sections of the embryos, and proficiency in manipulating stains to show up the cell walls and nuclei to the best advantage for microscopic examination. Next there is needed the ability to interpret correctly what is observed under the microscope. One might make a drawing of every stage encountered, even repeating many times what appears to be the same stage, and particularly to watch for mitotic figures and to note carefully the planes of the spindles. These division figures are most important in determining the Type to which the plant under study might belong, since it is in this manner that one decides whether the terminal or the basal cell of the two-celled proembryo divides first and in which plane. The drawings may then be arranged in the proper sequence and each original cell and its derivatives may then be traced from one stage to the other and the latter may also be traced backward to their origin. The origin of the hypophysis initial or hypophysis cell, if either is present; the origin of the epiphysis, if it occurs; the contribution of the basal cell to the construction of the embryo proper, if any; the origin and development of the epidermal initials; the origin and growth of the hypocotyl; the origin and development of the root tip initials—all these and many other embryological features may be discerned by a careful study and comparison of the drawings by themselves and by comparison with published descriptions of the same stages in related species whenever such might be available. Determination of the Type is ordinarily based upon the first four proembryonic cell generations, but a complete series of

stages of the later phases of development are usually required for proper assignment to a Variation. Keys for the determination of the various Variations under each Type have been presented elsewhere (1); these keys, it should be noted, do not represent the limits of those which might eventually be recognized, so that one is at liberty to propose new Variations if these are so characterized that others may understand the features on which they are based.

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AIR-SPACE TISSUE IN PLANTS

H. B. SIFTON

University of Toronto

In an anatomical survey of the plant kingdom one is forcibly impressed by the widespread occurrence of intercellular spaces. They are particularly characteristic of parenchyma, and vary from the common narrow passages where cells seem to have pulled apart at their corners, to the irregular spaces of the spongy mesophyll of leaves, and the large chambers common in aquatic plants. These spaces usually contain a mixture of atmospheric gases. Their occurrence in the tissues of plants is so general that it constitutes the typical condition, at least in the higher groups, and they have reached a high stage of development even in some of the more massive Thallophyta. This situation, which contrasts rather sharply with that in the animal kingdom, opens interesting problems from the standpoints of development, distribution and function.

In view of these facts, it is not surprising that observations on air-containing tissues are widely dispersed through botanical literature. Morphologists, physiologists, ecologists and students of special plant groups have all been interested. The present object is to bring together the separate discoveries that have been made, so that their conjoint significance may properly be assessed. An inspection of the literature impresses the reader with the great trend toward specialization in modern science. The students of air-space tissue in non-vascular and in vascular plants have, almost without exception, kept their work in separate compartments, and, indeed, within these divisions a similar tendency toward isolation is observable when one reads, for example, the results obtained with algae and with Bryophyta. In attempting to bring the discoveries together, the lower plants will be dealt with first, and later the more advanced will be given consideration.

THALLOPHYTA

Fungi

The air spaces of algae and fungi have received little attention compared to that devoted to comparable structures in higher plants. They are by no means absent from these groups, however, and

detailed study, particularly in the fungi, might lead to interesting conclusions, for the lack of photosynthetic production of oxygen and elimination of carbon dioxide in the latter plants must make the air-space conditions very different from those in green plants. The hollow stems, with or without felted hyphae, characteristic of various massive fruiting bodies, and the spongy pseudopar-enchyma of others, are used for identification, but I have seen little reference to their mode of development or their physiological signifi-cance.

In a few cases attention has been directed to the development of cavities in which asci or basidia are borne. In *Psalliota* (54) the primordium of the fruiting body is at first solid, but the hymenial primordium, which differentiates when the button is about a millimeter tall, soon pulls away from the tissues beneath, forming an annular cavity in which the gills with their basidial hyphae develop. In *Lycoperdon* (69) the hyphae in the interior of the young fruiting body pull apart in various places, forming cavities which contain very loosely woven hyphae with many broken ends. These cavities increase greatly in size as growth proceeds, and become lobed. The hyphae of the surrounding tissue grow to-ward the cavities, so that they appear bounded by palisade tissue. Basidia eventually develop from the hyphae of this palisade layer. It would appear that comparable developments occur in other Gasteromycetes and in those Ascomycetes that have perithecia. In fact, on looking through a modern text, such as that published by Smith (136), one is struck by the fact that in every case where asci or basidia are borne internally they are found projecting into rather large spaces previously formed. It would be interesting to know the composition of the gas contained in these spaces, and to investigate the mechanism by which ascogenous, basidial and other hyphae become oriented so as to grow into them. The fact that the asci or basidia occur only in such cavities or on free surfaces is also of interest. Even in a form like *Lophodermium*, a parasitic fungus whose asci are formed within the tissues of the host, an ascocarpic cavity develops as a preliminary to the production of asci (62).

Algae

In algae with massive bodies, intercellular spaces are commonly filled with the gelatinous substance of swollen cell walls. This

jelly is sometimes displaced by gas, however, even in forms not possessing special pneumatocysts. In this connection Oltmanns' (101) description of *Chorda* may be noted, in which he says the whole tissue mass encloses a central cavity filled with mucilage, or often with gases. Here and there diaphragms of proliferated hyphae cross the cavity. In *Enteromorpha intestinalis* also, accumulation of oxygen in the tubular plant body has been remarked (56).

The bladder-like floats that serve as supports for the fronds of numbers of the Phaeophyceae have become well known through the work of several investigators. They are plentiful among the Fucales and Laminariales, and are filled with gases.

In the Fucales rather careful morphological studies have been made of the bladders of *Fucus*. They develop near the growing point, in the medulla of the frond, and are said to be more numerous in plants of deeper water and in those growing in quiet places (111). First, a local thickening is observable, due to swelling of the gelatinous part of the cell walls. Then gas bubbles appear in the gelatinous matrix and enlarge, pushing the filaments of the medulla toward the outside (110). Richard (111) says the walls of these hyphae dissolve, a process not noted by the other investigators. The mature cyst is, at any rate, lined with a felted layer of filaments resembling the medullary hyphae (110, 166). Outside this are layers of ordinary cortical cells with their rather thick walls, and Richard described hyphae growing into the cavity from these cells. The outer cortical layers about the cyst are of thin-walled cells, gradually grading into typical cortical cells above and below, and the epidermis is normal and unmodified. It is emphasized (110, 111) that the gas in the bladders has no direct contact with the outer atmosphere.

Other members of the Fucales show minor differences. In *Ascophyllum nodosum* the main bladders are said to form early in May near the growing point, but smaller, secondary ones may form from time to time (80). The much divided, felted lining of *Fucus* is replaced in *Halidrys* and *Cystoseira* by a lining of parallel filaments, mostly unbranched (166). In *Halidrys* the bladders are divided into compartments by plates or cross walls of medullary parenchyma (110, 165, 26) with columns of long-stretched fibres uniting them (101), and *Halidrys dioica* displays every intergrade

between normal flattened fronds and well developed air bladders (27). Wille (166), who did not see the hyphae growing into the cavity of *Fucus* as later described by Richard, did describe short hairs extending into the space in *Ozothallia nodosa*. A similar production of hyphae was observed (27) in *Halidrys*. Such dissimilarities are important to the student of causal and experimental morphology, providing material with which he may work.

In the air-containing tissue of the Laminariales a series of forms with increasing specialization may be observed. Mention has already been made of the condition in *Chorda*, where gas may or may not have displaced the gelatinous matrix of the medulla.

In *Alaria fistulosa* the medulla of midrib and blade forms a series of distinct chambers (132), and their development is described as follows (66) : Long ragged slits appear along the median line of the pith. The tendency is to form one rift, rather than a number of small ones side by side. The rifts at this stage are in a vertical series, separated from each other by masses of hyphal chains with small clefts. The side walls of a large rift bulge outward slightly and are lined with torn hyphal chains. The ends are sharply angled, as if torn. The first true chamber, seen in slightly more mature tissue, has ends less sharply angled, and walls with fewer hyphal chains. The septa between chambers are more compact, and when they are mature there is no sharp line of demarcation between inner cortex and septum. The mature chamber has walls practically free from hyphae, and ends no longer acutely angled.

Miss Kibbe suggests that the explanation of this series of developmental stages lies in the strains produced by differential growth. The plant is supplied with the elongated, sieve-tube-like cells found frequently in brown algae. These cells, having finished their growth, become stretched, she believes, by the rapid division and growth of cells of the inner cortex. As a result of the consequent strain, the cortical cells are forced out of alignment, causing the walls of the midrib to bulge, and tearing the hyphal strands that extend horizontally across the medulla. Longitudinal chains are eventually broken also, and between the breaks, *i.e.*, in the regions of the septa, the tension is somewhat relieved. Continued divisions of cortical cells enlarge the chambers and tear the pith, but the cells of the septa, where longitudinal strain is reduced, are able to withstand the lateral strain.

The hypothesis fails to explain the disappearance of medullary hyphae from the chambers. Even the fracture of the longitudinal ones requires more explanation if we are to assume the presence of inextensible sieve tubes that reduce longitudinal growth to a minimum. No experimental evidence was given to indicate great brittleness on the part of the hyphal walls, or especial toughness and inextensibility in the sieve tubes. Further examination of the development of these chambers would be interesting, in the light of more recent work on air-space tissue in higher plants.

It should be observed that Miss Kibbe is not alone among phycologists in suggesting such an origin for air spaces. A similar suggestion was put forward in 1885 with respect to the bladders of *Lessonia ovata* when the pushing apart of cells to produce the cavity was ascribed (46) to strong growth in area of the epidermal cells about the point concerned.

More specialized than the air-space tissue in *Alaria* are the gas-filled floats of *Macrocystis* and *Nereocystis*. The former (9) has a peculiarly developed, compound frond on which the pneumatocysts are borne in definite positions. Large primary fronds are produced, each with a thick cord running along one side of the lamina. Near the cord, perforations in the lamina occur which, by extension, cut the broad blade into a series of secondary fronds, each joined to the lateral cord by a short stipe. Of these the first few cut off (usually two) have no air bladders, but each of the succeeding ones develops one near its base. Two further observations on production of the bladders have since been made (95). One is that the primary frond may pass through a rest period and become rejuvenated, producing, as a result, more bladderless secondaries. The other is that a bladder may be formed in the growing region of a primary frond within the terminal blade, but when this has taken place further growth and cell division at the apex of that frond ceases. Where such bladders occur at all, they are usually fairly abundant and indicate general deterioration. It would thus seem that the production of a bladder, whether on a primary or a secondary frond, is associated with a limitation of growth in the tissues beyond it.

Development of the bladders was described (164) in 1885, and is not unlike that of the air tissue in *Alaria*. First, the intercellular jelly of the medulla swells in the locality where the bladder is to form. Then little air spaces appear here and there in the jelly. These

spaces grow and unite, forcing the loose medullary tissue toward the periphery, so that there is finally one large gas-filled space, crossed at intervals by individual hyphae. A later account (116) agrees with the above, except that the tissues of the pith are said to be gradually resorbed, so that in an old float this tissue has completely disappeared. A cambium produces secondary growth about the bladder.

The course of float development in *Nereocystis* is described as similar (81), there being at first rifts in the jelly, then a bubble increasing rapidly in size. The pneumatocyst wall has two cambia, an outer and an inner one. The inner is not found in other parts of the plant.

Physiologists have been interested in the pressure and constitution of the gas contained in algal pneumatocysts. The earliest analysis I have seen (114) gave pure nitrogen as the content of the bladders of *Fucus*, but this did not remain unchallenged. Wille (166), in bladders of Fucaceae under natural conditions in daytime, found 35–37% oxygen. After ten hours exposure to air the bladders contained only 20.7–20.8% oxygen, and ten hours in darkness reduced the proportion to 2.7%. A similar increase in oxygen content in daytime for the Fucales was obtained by Colla (20) who found this gas increasing in the forenoon and decreasing in afternoon. No carbon dioxide was identified by any of the above workers, and in this their results were in agreement with those obtained on gases from the vesicles of a number of Australian forms (78).

A group of American workers have published a series of reports on gas relationships in the floats of *Nereocystis*. The first of these (168) reports carbon dioxide varying from an average of 0.293% in daytime to 2.503% at night, while the corresponding percentages of oxygen were 12.304 and 10.906. Later papers, however, report that frequently the carbon dioxide is not present in appreciable quantities (70, 113), and the percentage of oxygen may be considerably higher. An interesting point was the discovery of carbon monoxide in the bladders (70, 113, 71). It was found in concentrations of from 1% to 12% (70). Its rate of formation was not influenced by light, but oxygen in the bladder was necessary for its production (71). Since it could not be produced by autolysis or fermentation of ground up plants (71), and since culture methods indicated bacterial sterility of the bladder cavities (112), it was concluded that the gas was a product of respiration of the plant itself.

The gas pressure in the bladders was found to vary from 124 mm. above atmospheric pressure to 316 mm. below. The pressure was higher in floating than in submerged bladders, and higher in frondless plants, and it decreased at night (41). Later, studies of composition and pressure changes in the gas of single specimens (113) led to the conclusion that the changes in pressure may be due to a change in oxygen content, with which they were always found to coincide.

BRYOPHYTA

Hepaticae

The conspicuous air chambers in the gametophytes of Marchantiales have attracted the attention of botanists from remote times. The members of the order have been classified into three types (36), based on the appearance of these chambers in sections of the mature thalli. In the *Riccia* type the chambers are in a single layer in the dorsal part of the thallus, and form rather long, more or less vertical canals, usually very narrow but sometimes broader. The *Marchantia* type also has its chambers clearly in a single layer, but they are broad, flat and polygonal, roofed by an epidermis, except for the central pore by which each communicates with the outer atmosphere. With few exceptions simple or branched photosynthetic filaments grow up from the floor of the chambers. On examining a section of the third or *Reboulia* type, one sees many irregular polyhedric chambers, in two or more layers, and often without but sometimes with cellular filaments or plates extending into them. That these chambers, whatever their arrangement, perform a useful function in placing extended areas of photosynthetic tissue in contact with air that is not excessively dry, is a thesis on which all can agree.

The course of development of the chambers presents points of interest and has given rise to some controversy. In this as in so many other problems of development, initial observations were made by Hofmeister. He held (59) that in *Marchantia polymorpha*, at the first appearance of the cavity, a single layer of cells is separated from the rest and lifted up by divisions in the cells that are to form walls between the cavities. He apparently did not observe the earliest stages, but clearly concluded that the origin of the chamber was endogenous and schizogenous. Kny (68) reported a schizogenous origin also for the canal-like cavities of the Ricciaceae but found the neighbouring rows of cells separating from without inward. Some

years later, however, in Leitgeb's comprehensive studies of the liver-
worts, he put forward a quite different concept of the origin and
development of the air chambers in both Ricciaceae and Mar-
chantiaceae (75, 76). He concluded that there was no separation
of cells previously joined and no secondary lifting of the chamber
roof from the underlying tissues, and his account of the origin of the
chambers may be summarized as follows. At a point where four
cells of the uppermost layer come together, the vertical growth of
their lateral walls is less than in other places. This causes a little
pit to form at the junction of the four cells. Next, a division
parallel to the surface of the thallus occurs in each of the four cells,
separating the part that forms the side of the pit from the rest of
the cell. There is now a small pit surrounded by four cells, though
no splitting apart has occurred. Successive horizontal divisions in
these cells add to the side walls of the pit, which at the same time
may be widened by extension and division of the cells in the thallus
beneath. The roof which covers the broad chambers of forms like
Marchantia has its beginning in oblique divisions of the top cells of
the side walls. This description had the advantage, in Leitgeb's
view, of bringing the air chambers into harmony with the depressions
that contain sex organs. These organs, when very young, project
above the thallus, but they later become overgrown by the surround-
ing tissues.

For more than 25 years workers on liverworts seem to have been in
unanimous agreement with Leitgeb (42, 15, 16, 17, 45, 13), but in
1907 Barnes and Land (3) joined issue with him. Describing in de-
tail the growth of chambers in a number of genera, they found them
originating invariably by the splitting apart of cell walls within the
thallus. The great majority of later writers agree that, in the forms
they studied, the chambers have originated by splitting, beginning
either from the outside (25, 100, 104, 53, 102, 103,) or from inside
(107, 143, 30, 52, 102, 133, 48) the tissues. Perhaps an indication of
the cause of some of the divergence of opinion as to the place where
the splitting starts may be gathered from Evans' (36) work on
Grimaldia fragrans, in which he found that the splitting was some-
times from within, sometimes from without. Two workers (58, 7)
since 1907 have reported forms in the Ricciaceae developing as
described by Leitgeb. Commenting on the latter paper, Evans
holds that the drawings are not convincing and that evidence

against splitting from without was not presented. In the former, four species are described as differing from the description of Barnes and Land. Of these, three were studied only from soaked herbarium specimens, and the drawing of the fourth suggests splitting. Campbell, however, in a later edition of his text on mosses and ferns cites these papers and that of O'Keefe (100) as substantiating Leitgeb's hypothesis.

Once the air chamber has been initiated, it grows by active enlargement and division on the part of the surrounding cells. On this all are agreed and no one has found any evidence of enlargement by the death and disappearance of cells, as is seen in the lysigenous spaces of higher plants. The pronounced tendency toward cell growth and division about the young air chambers is so general in these forms as to merit special mention. In many species it is indicated not merely by the increase in size of chambers but by the growth of filaments (144, 15, 3, 25, 87, 52, 102, *etc.*) or plates (96, 36, 30, 104) of chlorophyll-bearing cells into them. A number of writers have reported these plates as forming complete partitions across the chambers in the forms they studied (16, 13, 124, 133).

There seems little occasion for doubt that chambers of the *Riccia* and those of the *Marchantia* group are fundamentally similar, their differences being explainable on the basis of different growth reactions on the part of the surrounding cells after the chambers have been initiated. In fact, some species of the *Riccia* group have chambers not unlike small ones of the *Marchantia* type. Juel (64) describes in *Riccia Bischoffii,* in the region of the midrib, chambers in the form of narrow canals, each bounded by four rows of cells. Out in the wings, however, the canals become progressively wider as the distance from the midrib increases, till the broader ones become spaces surrounded by 6 to 15 cells. He states that the broader canals on the flanks arise from the greater radial growth in that region.

Evans (36) names 12 genera among American liverworts alone, with chambers in two or more layers. The deeper ones have been supposed by some (16, 124, 133) to be parts cut off from the primary chambers by the plates growing in from floor and walls, a plate in such a case growing across the chamber and fusing with the wall on the other side. Others disagree with this. It seems clear (3, 30, 53, 36, 102) that the secondary chambers connect up with the

primary dorsal ones, and the tendency to think of them as merely extensions of these, produced by extension of the splitting to deeper cells of the thallus (30, 53, 102), would, if correct, place the air spaces in liverworts more in line with those in higher plants.

On the development of air-space tissue in liverworts other than *Marchantiales*, little has been written. In Jungermanniales the assimilatory tissue is spread out in thin plates, and air chambers are lacking. Of the Anthocerotales in general it is said (3) that triangular and quadrangular spaces occur in both sporophyte and gametophyte, similar to those typical of the parenchyma of vascular plants. In addition, many members of this group have large lacunae in their gametophytes. Of the 291 species described in Stephani's *Species Hepaticarum* (145), 130 are described by the term *frons cavernosa* or *costa cavernosa,* indicating the presence of such lacunae.

From the standpoint of causal morphology, the air chambers of liverworts have been accorded just sufficient attention to indicate that further experimental work might produce results of consider- able interest. The genus *Dumortiera* is interesting in that typical air chambers are normally more or less completely eliminated from the mature thallus. In *D. hirsuta,* Coker (19) noted that indi- viduals growing on a gentle slope where spring water kept them continually wet, had no sign of chambers. Those growing on moist sand in a cave, however, produced near the growing point well formed chambers with irregular pores and scattered papillae. The chambers disappeared as the tissues became more mature, but the drier conditions in the cave clearly favoured their development. In *D. velutina* and *D. tricocephala,* Ernst (35) reported the upper epidermis completely lacking. The chambers were consequently roofless, but their side walls developed and papillae projected from their floors. In *D. trichocephala,* even the side walls and papillae were finally eliminated from older parts of the plant. This writer also noted that the amount of reduction was influenced by water relations. Campbell (14), in *D. trichocephala* from Hawaii, found no rudiments or vestiges of air chambers. In other species of the genus, chambers were evident near the tip, and the side walls might persist, though the upper epidermis always dis- appeared. Insofar as the action of water is concerned, Campbell found in *D. calcicola,* which grew in dry places, well developed

chambers near the tip, and side walls and papillae remaining in older parts. He emphasized that moisture was not the only controlling factor, however, as two species growing together and under identical conditions with respect to moisture had different degrees of suppression of the chambers. Experiments with *Dumortiera* might suggest the mechanism through which moisture acts on this very sensitive tissue, and thus add to our knowledge of cell ecology.

Two researches on *Marchantia* and its air chambers give ground for hope that here also the experimental morphologist might find material for investigating the effect of water on these structures. As long ago as 1893 it was observed (117) that in submerged plants there were in many places no chambers, and where they did develop they were shallower than normal and contained a less luxuriant growth of assimilatory filaments. Only a few of the chambers were filled with air. The report of the inhibiting effect of submergence in water on air-chamber production was confirmed in the latter of the two papers (40), but this author was inclined to think that the limiting effect of the presence of water was an indirect one. He noted that the spaces that did contain air were normal, though the whole plant was submerged in water during their development. The air chambers of *Fegatella* are also affected by submergence (28), though perhaps to a lesser extent.

Cutting down the light intensity has been found to have an inhibitory effect in three genera investigated (5, 28, 39). Various colours of light were tried on *Marchantia* (39), and while in red light the chambers were normal, in blue they contained no assimilatory filaments, and in green almost none was produced. This is interesting in the light of Teodoresco's finding that chambers and filaments were very luxuriantly produced in concentrations of 1.5–2% carbon dioxide and were much reduced when thalli grew in the absence of this gas. Perhaps their development is bound up with photosynthesis.

Musci

The moss gametophyte seems to be entirely lacking in schizogenous spaces such as we have described for the liverworts. There is no suggestion that the air-enclosing outgrowths on the upper side of *Polytrichum* leaves are anything but outgrowths. We have here, however, active growth and division of chlorenchyma cells in contact with air, entirely comparable to that concerned in

the enlargement of liverwort air chambers, and in at least one species of moss (160) this growth was found to be inhibited by darkness, as it was in some liverworts. Moss gametophytes in general, like those of Jungermanniales, are characterized by the arrangement of their green tissue in thin plates, a form of body not conducive to air-space development.

The stem of the moss gametophyte is a column many cells thick, and the central part of this sometimes contains cavities. Such spaces were found by Lorch (77) to be normal in at least two species, of different genera. In *Campylopus polytrichoides,* he was able to state that the cavity was lysigenous in nature, produced by the resorption of the central strand of the stem. Remains of the dead cells were still observable in the cavity at maturity.

The sporophytes of mosses are commonly green when young and exhibit a many-layered assimilatory tissue comparable with that of the vascular sporophytes of higher plants. In this tissue, air spaces among the cells are present in most if not all species (72, 49, 50, 158, 159, 61, 77, 162, 163). The small interconnecting spaces described by Haberlandt (49) in the chlorophyll-bearing tissue of some forms, opening to the outer air through the stomata, were found often to extend to the apophysis and, even, in some cases, to the seta (158, 159, 61, 163). Vaizey (158) found such spaces present also in the seta of *Atrichum undulatum,* a form without stomata. Lorch (77) speaks of spaces in the seta as characteristic of the families Polytricaceae, Dawsoniaceae and Buxbaumiaceae. He looks on these as capillary conductors of water, however, and not as air spaces.

In special parts of capsule and apophysis it is very common for these spaces to enlarge greatly by rapid growth and division of adjacent cells, and in this way are produced the large lacunae so common in the chlorophyll-bearing tissues of moss capsules (72, 49, 159, 61, 77, 76, 162, 163). Often the separation, growth and division of cells to produce these lacunae proceeds with almost diagrammatic regularity. It has been described in detail for *Polytrichum Juniperinum* (162).

Lysigenous spaces may sometimes be present in the seta. Vaizey (158) observes that a large space of this category is found in the centre of the leptoxylem in *Splachnum sphaericum,* and while Lorch (77) classified the spaces he found as schizogenous, van der

Wijk (163) states that they become larger through collapse of cells, which observation, if correct, would place them in the other category.

VASCULAR PLANTS

In the sporophytes of vascular plants, air spaces are so common that there is a tendency to take them for granted. A survey of the literature fails to find a plant in this group recorded as without them.

Small Air Spaces of Unspecialized Tissues

The small intercellular spaces that are the forerunners of all air spaces in primary tissues, appear very close to the apical growing point. For details of development in this region we are indebted to the work of Schüepp (129) and Priestley (108). The rapidly dividing cells at the growing point have no intercellular spaces, being plastic in nature and closely pressed together. Very soon, however, differentiation begins, and a change from rapidly dividing, non-vacuolated cells to more slowly dividing, vacuolating cells is accompanied by the appearance of small spaces, as if the cell walls had become elastic and the enlarging cells had rounded up and pulled apart at the corners. At first these spaces are filled with liquid, but very soon the liquid is replaced by air, and at the same time, according to Priestley's work, the change from a slowly vacuolating, dividing cell to a rapidly extending cell takes place. The small air spaces have by this time united to form a continuous system which, in the aerial shoot, connects with the outer air through the stomata. In many tissues the aerating system ceases development at this stage, but not infrequently larger spaces develop in various ways from this beginning.

Before passing on to consider the larger spaces it is necessary to discuss a problem in connection with these first stages of development, a problem that presents itself when the constitution of the cell wall and the composition of the lining layer of the air spaces is investigated.

Meristematic cells are separated from one another by a middle lamella of a pectic nature, and inside this pectic envelope each protoplast has completely surrounded itself with a thin primary wall of a combination of cellulose and "pectin" (156). The word "pectin" has often been rather loosely used in the literature to indicate soft pectic substances. Microchemical evidence indicates the presence

of pectic acid rather than true pectin in young growing points (23, 88, 135), but in the present connection the difference is not important.

In the decade from 1880 to 1890 there was some controversy as to the lining of intercellular spaces. Earlier contentions that the substance was protoplasm (118) have been overcome, and present workers are in agreement with those (122, 167, 83) who held that, except in special cases where some lignification or suberization may have taken place, the lining is composed of the pectic substance of the middle lamella. The original mistake was perhaps due to the fact that near the growing point microchemical reagents used to test for cell wall substances usually fail to react characteristically, a fact held by Priestley's school to be due to the presence of fats and protein in the walls in this region (156).

The spaces that form at the corners of cells might, in harmony with the above, be held to be due simply to the splitting apart of the middle lamella at these points, but when one considers in detail the formation of the new wall at cell division the insufficiency of this simple explanation becomes evident. The meristematic cell about to divide is completely surrounded by two layers, the middle lamella and the primary wall. After cell division a middle lamella is laid down in the cell plate separating the two daughter cells, and a layer of primary wall forms on each side of it. The new middle lamella joins up at its edges with the side walls of the parent cell, but between it and the old middle lamella of the parent cell is the thickness of that cell's primary wall. It is at such a corner that a small intercellular space forms when the cells begin to vacuolate, and such a space when mature is always completely lined with middle lamella substance in continuity with the middle lamellae of adjacent cells. There is no evidence of a break in the pectic layer, and a problem arises as to what becomes of the primary wall layer that originally separates the newly formed middle lamella from that of the parent cell.

Workers in Martens' laboratory (65, 84, 85) undertook a series of developmental studies with attention directed to this point, and in primary and secondary tissues of a number of forms they reported the following. When a cell divides, the new middle lamella is separated from the old one about the original cell by a layer of cellulose wall. The free ends of the new middle lamella then

spread or thicken so that in transverse section a triangular mass is seen. This mass enlarges and becomes a cavity, triangular in section, surrounded by pectic substance of the middle lamella. As the cavity grows, the cellulose wall between it and the middle lamella of the parent cell breaks or is dissolved, and the cavity thus becomes an intercellular space. Sometimes the older middle lamella splits opposite the expanding end of the younger, and in such cases the two spaces fuse when the cellulose has been broken down. Confirmatory evidence has been obtained (109) from macerations of collenchyma, sclerenchyma and the parenchyma of young internodes.

Larger Air Spaces in Primary Tissues

Although many tissues are characterized by the above type of air space at maturity, it is a common occurrence for development to proceed further, culminating in larger spaces than those described and often in the large lacunae characteristic of water plants. Viewed morphologically, plants and tissues that have attained more advanced types of air spaces appear to belong to two separate groups, and while much still remains to be done in causal and experimental research, it seems, in the present state of our knowledge, that this classification depends on rather pronounced differences in cell metabolism.

The least accentuated example in the first group is perhaps to be found in the spongy mesophyll tissue characteristic of large numbers of leaves. In this tissue the spaces described above continue to increase in size. Instead of the turgid cells pushing their rounded contours into the sharply angled spaces, it comes about that the walls are drawn in at those points where they are not in contact with other cells, and the spaces between them become rounded out. In more pronounced cases the tissue is finally composed of extremely irregular cells maintaining contact with their neighbours by means of arms projecting in various directions, so that the tissue becomes a loose air-containing meshwork. In some leaves this tendency extends also to the palisade cells (94, 150, 157), whose sides are finally connected only by projecting arms.

It has been observed (150) that in leaves with both palisade and spongy parenchyma, cell division ceases earlier in the cells of the spongy tissue, and the intercellular space system depends on the ex-

tent of the "stretching stage", which, in turn, is initiated during the development of the stomata. This fits in well with Priestley's observation that the period of rapid cell expansion in stem tips begins with the entrance of air into the then minute spaces.

Satisfying experiments with leaves to show the mechanics of the production of the spongy parenchyma cells are lacking, but some researches on the relative volume of air spaces in different leaves and under various conditions are of interest to students of causal morphology. In measuring the size of the air spaces the majority of workers have used methods based on that originated by Unger (157) in 1854. He estimated their volume by comparing the weight of pieces of tissue in their normal condition with that attained when the spaces had been injected with water by the use of an air pump. Two refinements of this method may be mentioned. Kisser (67) pointed out that the use of the air pump sometimes results, unless extreme care is taken, in the harming of delicate tissues, and recommended the use of a centrifuge as a subsidiary method; Nius (98) used turpentine instead of water for the infiltration. Another method, in which the areas and perimeters of spaces as seen in microscopic sections are measured, has also been used (106) but less frequently. An idea of the proportion of air by volume in normal leaves may be obtained from Unger's results. Among the 41 species he investigated, he found a minimum proportion of spongy parenchyma (141) and a larger volume of a maximum of 713 parts per 1,000 in *Pistia texensis*. Later investigators have found that conditions have a significant effect on the volume of the spaces. Leaves grown in shade have a larger proportion of spongy parenchyma (141) and a larger volume of air spaces (142, 147, 128). The air-space volume seems to vary with the water supply (98, 128), but to a much lesser degree in some plants than in others (98). Evergreen leaves of the temperate zones were found to have a smaller air volume than deciduous (98), and in soft evergreen leaves this volume decreased in autumn (128). In a number of leaves it has been found that spaces develop well in white or blue light, poorly in red, and very poorly in green or in complete darkness (149).

We may now proceed from the spongy tissue of a normal green leaf to consider the more extreme modifications exhibited by such forms as *Typha* and *Juncus*. The young *Typha* leaf has in the

beginning a homogeneous parenchyma (38), the cells of which grow and pull apart much as has been described, but the process proceeds further than in most leaves. The final result (92, 139) is that in the leaf-sheath there are large lacunae filled with a very loose tissue of long-branched cells, very thin-walled, and mostly free from the lacunar walls, while in the blade are seen typical lysigenous lacunae from which the loose tissue has disappeared. Here and there in both sheath and blade are places where the metamorphosis of cells has not gone so far and diaphragms are left. Those in the sheath are composed of cells similar to the loose ones in the lacunae but with their vertical arms short and their horizontal ones remaining attached to the sides. In the blade the diaphragms are composed of short-armed cells, thick-walled in the basal diaphragms but thin-walled near the leaf tip. A somewhat similar situation is reported in *Juncus inflexus* (32) which may have on the same root, stems with continuous pith, with pith interrupted at certain regions, with pith almost entirely disappeared, or with pith partially disintegrated leaving a ring of canals. In Pandanaceae (139), as in Typhaceae, some of the spaces contain branched cell tissue and some do not. Such stellate cells are also to be seen in adult spaces in *Scirpus* (137) and could doubtless be found in other forms.

Large lysigenous air spaces are common in water plants and are found in numbers of land plants as well (60, 127, 57, 88, 165). Some attention has been paid to the causal aspect of their development. As long ago as 1855 Leitgeb (74) was interested in this aspect and his paper includes an outline of literature back to Malpighi and Grew. He thought the cells became star-shaped by secreting air into the small spaces and enlarging them while adhering to each other at the points where their walls were in contact. Duval-Jouve (31, 34) came next with the idea that the cells were pulled and stretched by continued growth of the surrounding tissue, and in this way the arms were drawn out at points where the walls of adjacent cells adhered. He said that true lacunae were formed because the stretched cells could not keep pace with the growth of the surrounding layer, and their torn remains could be seen adhering to the sides of the mature canal. The same conclusions seem to have been arrived at independently by some later workers (146, 134), and Newcombe (97), after an experimental

study with 24 different plants, concluded that the tension of tissues is a factor in limiting the life-period of the cells which normally collapse in cavity formation. He found, however, that although the development of a cavity could be deferred by artificially preventing extension of the surrounding tissue, the cells that normally collapsed would finally die without this extension.

The hypothesis of the shaping of the armed or stellate cells and their final rupture and death by the pull of a growing ring of surrounding tissue does not fit with the fact that often lysigenous spaces are surrounded only by layers of soft tissues that would be easily drawn inward and could not produce the required tension. McPherson (88), from his experiments on cortical spaces in roots, arrived at another hypothesis which is more in accord with the facts as they are known at present. In the roots of *Zea Mays* the series of morphological changes leading to the formation of an air space was the usual one. First, the cortical cells passed through the normal period of rapid vacuolation with small spaces at the corners, and this period, with the cells turgid, pressing against each other and rounding out into the spaces, endured throughout the time the root was increasing in diameter. Later, the cells in the region where an air space was to form, began to collapse. Where the walls of adjacent cells touched, they adhered, and thus the collapse of the cell gave it the peculiar, irregular shape, with projecting arms. There was here no question of the arms being drawn out by growth of the ring of surrounding tissue, for such growth had previously ceased. Later the protoplasm of these cells became progressively thinner and finally disappeared, the soft cell walls collapsed completely, and a lacuna resulted.

The hypothesis was that the active protoplasm of these cells used up food materials more rapidly than they were being supplied by the conducting system, and then destroyed itself by respiring its own proteins, as is commonly done by starving cells. It was found that immersion in water, or any other procedure that brought about a lowering of the oxygen supply, produced larger lacunae, a finding in agreement with those of numerous workers (21, 22, 99, 8, 29, 12) and one to be expected according to McPherson's hypothesis, if one remembers the especially rapid breakdown of food material in anaerobic respiration. In fact, the concentration of oxygen about the root could be increased to the point where no lacunae would be

produced. In experiments with a number of other plants it was shown that each had its own critical oxygen pressure, below which lacunae would form.

One more condition was essential for the normal formation of lacunae. They developed normally when the cell walls were composed of cellulose and pectic acid, as revealed by microchemical tests, but not in those where the pectic constituent was calcium pectate. In the latter case cells died and their protoplasm disappeared, but the hardened cell walls retained their normal shape.

Later a similar origin was suggested for the lysigenous spaces in the leaves of *Ledum groenlandicum* (135). In this case the cells reach an abnormal size before collapsing, actually pushing out the soft lower surface of the leaf into little mounds. Some evidence is produced that this rapid increase is due to an increase in permeability to water on the part of the protoplasm. Such permeability increase would continue in protoplasm undergoing autolysis, until complete permeability replaced semipermeability and collapse resulted.

This brings up to date the account of investigations of lysigenous spaces. Whether the early stages of their development are fundamentally the same as those producing normal spongy mesophyll may some day be determined by physiological experiment. The morphological similarity is very close.

With regard to the development of schizogenous lacunae, one feels a need of further investigation. Even a casual inspection reveals the profound difference between the reaction of the cells concerned in their production and that of those forming the lysigenous spaces. In both cases the beginning is the same, small airfilled chinks developing among the cells, but beyond that the similarity ends. The cells that are to form the schizogenous lacunar tissue act as if stimulated by contact with the air, growing and dividing rapidly. Thus the original small spaces, triangular or quadrangular in cross section, become large smooth-walled cavities or canals, walled by the progeny of the three or four cells or tiers of cells that originally enclosed them.

In some cases the cell divisions producing the lacunae follow a course of extreme regularity. Sauvageau (120, 121) has recorded this for the leaves of *Zostera*. In this plant the very young mesophyll is of cells elongated transversely of the leaf. These

draw apart at the corners, leaving small spaces. At the same time
a very small cell is cut off from one end of each elongated cell,
adjacent to the space. Both elongated cells and small ones now
undergo a series of divisions. The daughters of the elongated cells
form the sides of the lacunae, while those of the small ones form the
diaphragms, one cell thick with small and usually triangular pores.
He listed seven other genera of aquatic monocotyledons with similar
development, on one of which (*Enhalus*) his findings have been
confirmed by later work (24). Others had spaces of less regular
growth.

The pronounced tendency toward growth and division on the part
of cells touching spaces in plants that form schizogenous lacunae
is well illustrated in the peculiar diaphragms of *Nuphar*, the
development of which has been observed repeatedly (153, 47, 11).
The air canals are at first uninterrupted by cross diaphragms, but
certain cells of the walls form swellings which project into the cavity,
branch and divide rapidly, forming masses of loosely arranged cells
across the canals.

In one case the development of large schizogenous spaces un-
connected with increased cell division has been suggested. Hall-
quist (51), in the pneumatophores of *Lobelia exaltata,* described
large cortical lacunae, apparently schizogenous, into the production
of which cell division seemed not to have entered. A study of the
development of these lacunae would be of interest. They are de-
scribed as having single layers of radially stretched cells between
them. In *Myriophyllum* an identical arrangement of tissues has
been described (161) as arising by tangential divisions of the
cells separating the spaces, followed by radial stretching.

Before passing from the consideration of lacunae in primary
tissues, something more should be said of the diaphragms by
which, in many forms, they are interrupted at intervals. These
diaphragms attracted the attention of earlier anatomists who re-
corded their presence in numerous genera and families (154, 18,
152, 33, 38, 125, 119, 44). When Le Blanc (73) stated, as an in-
dication that their existence does not depend entirely on aquatic
environment, that they are found only in Monocotyledons and the
related Nymphaeaceae, he had apparently overlooked one or two
papers. The great majority of plants in which they have been
found do belong to these groups, but they have also been reported
in *Utricularia* (152) and the Haloragidaceae (44).

The diaphragms assume a number of forms. In *Nuphar* (153, 154, 47, 11) and *Brasenia* leaves (125) we have the air canals occluded by loosely-packed masses of cells, outgrowths from the canal walls. In most plants with diaphragms, however, they have a definite tissue form. The diaphragms are most commonly one cell thick, but may have a thickness of two or three (34, 91) or several (33, 74) cells. In a number of cases they are traversed by veins (32, 33, 34, 73), and one writer (73) has suggested the support of these veins as one of their functions.

In most plants the diaphragms are perforated by large numbers of very fine pores. Sometimes the pores occur only at the corners where more than two cells were originally in contact, in which case they are presumably produced like those among the star-shaped cells of developing lysigenous spaces, the main difference being that the arms on the diaphragm cells usually remain quite short. Often, however, the cells draw apart, not only at the corners but at many other places, so that each cell appears in face view surrounded by numbers of short finger-like processes whose tips are in contact with the tips of similar ones of adjacent cells. This enlarges greatly the number of minute pores in the diaphragms, and incidentally raises an additional problem of development. We need to explain the pulling apart of cells at numerous places along their sides, while they closely adhere at intervening points. Goebel (44) suggested that the cells adhere by the thickenings of their walls, while remaining free to separate at the pits, a hypothesis not very popular among later writers who, however, have not succeeded in solving the problem.

The question of the contribution of environmental conditions to the development of diaphragm cells has also proved difficult. According to Le Blanc's account (73), a swelling and rounding out of young diaphragm cells gave rise to small angular spaces at their corners. This was followed by a shrinkage of cell bulk, perhaps due to evaporation into the spaces, and this shrinkage, together with a stretching of the whole diaphragm by growth of the surrounding tissues, accounted, in his opinion, for the final size and shape of the spaces. Snow (137) suggested that in *Scirpus validus* the formation of a diaphragm resulted from a retention of meristematic properties on the part of the cells of a layer, while those on either side ceased growth and became the slender stellate air-space cells.

She found (138) that a decreased rate of growth was associated with an increase in distance between diaphragms. In general, such a decreased growth rate followed a change from water to air or from high to low temperature. There were many exceptions, however, and satisfying results on the causal morphology of the diaphragms are still lacking.

Air Space Tissue from Phellogen

Cells of the phelloderm layers produced centripetally by cork cambium behave much as parenchyma cells, drawing apart at their corners to produce the characteristic, small, angular spaces. The cork cells, on the other hand, remain tabular in shape, their walls becoming suberized before they swell and round out by vacuolation, and so they usually lack intercellular spaces.

The complementary cell tissue of lenticels provides an illustration of special air-space tissue produced by phellogen. It is stated (26) that lenticels are found in all great groups of vascular plants and on all their organs, but generally only in those plants and organs with secondary growth. Trécul (155) observed their origin in 56 species and found them developing under stomata in all. They are not always limited to this position, however, and have been classified on the basis of their origin (26) into two groups, namely primary ones, developing early on a spot determined by an organ such as a stoma, root or bud, and secondary ones developing late at other points.

The development of a lenticel was followed and described more than seventy years ago (140). In *Sambucus nigra* the following stages were noted. Under a stoma in a twig, cortical parenchyma was found to extend to the epidermis, displacing the sub-epidermal collenchyma. The parenchyma cells of this group enlarged and divided, along with some of the collenchyma cells, so that the epidermis was stretched and a small lump appeared. The cells rounded up and thus produced intercellular spaces and became a mass of complementary cells. Inner layers became a cambium, producing complementary cells toward the outside and phelloderm toward the inside, and the epidermis was torn by this increased growth. In some other forms the same author notes that the phellogen is responsible for even the first steps in lenticel formation, and it is only after a layer of cork has been laid down that swollen complementary cells are produced.

In the mature lenticel, layers of complementary cells alternate with layers of cork, each cork layer sealing the lenticel until broken by expansion of later formed complementary cells. The complementary cells constitute the air-space tissue. Devaux (26) has noted that in some lenticels even the suberized cells of the closing layer round up, presumably before suberization, and produce spaces.

It seems undeniable that the production of complementary cells instead of cork cells is bound up in some way with abundant water supply. When a corky layer has closed the lenticel, the inner tissues are protected from water loss, and complementary cells are formed. When a twig is placed in water or in air saturated with water vapour, it is normal for so-called water lenticels to form with no closing layers but a continuous production of complementary cells. Devaux (26), who reported this, found it quite possible, by regulating transpiration, to change a water lenticel to an air lenticel and *vice versa*. On the other hand, it has been found that in the roots of cotton plants the lenticels became much more hypertrophied under boiled water than under tap water periodically aerated (148). If this reaction is general, it would seem that water, in addition to its direct effect, brings about a secondary stimulation of complementary cell production by cutting down the oxygen supply.

In a number of plants this tendency to produce unsuberized cells and air-space tissue under the influence of an aquatic environment extends to the whole of the phellogen. Of such plants at least 39 species have been investigated (86, 115, 105, 43, 63, 130, 123, 126, 6, 10, 131, 89), and numbers of additional ones could doubtless be found.

The cells of aerenchyma tissue so produced have been found to have no suberin in their walls (123), and often possess the further specialization that they assume definite forms and so produce a regular tissue structure in place of the irregular mass of cells and spaces in the air-containing portion of a lenticel. A relatively unspecialized type may be seen in *Sesbania* and *Neptunia* (115, 130, 89). The tissue here is irregular. The only advance over the tissue of complementary cells in a lenticel is seen in a tendency to elongation and sometimes branching on the part of the cells, which thus produce a rather more porous mass. The more ad-

vanced type, as seen in *Nesea verticillata*, has been well described
(126). The cells cut off from the phellogen are flat and oblong,
but soon round off so that their tangential faces are in contact
only over a small central part of each cell. Then certain individual
cells in each tangential layer push out bulges in a radial direction.
The result is that successive layers are pushed apart. The inter-
vening spaces become filled with air and are interrupted only by
the beam-like projections from the special cells, which hold the
layers apart.

   Submergence in water provides the stimulus for the production
of aerenchyma. Plants that normally produce it were found, when
growing in dry situations, to have none (123), but to produce cork
instead, and in *Nesea* (126), branches that bend over and dip in
the water form aerenchyma instead of cork on the submerged parts.
Seliber (131) has, however, pointed out from his work on *Jussieua*
that good nourishment and some aeration are also requisite.
Wholly submerged plants failed to produce aerenchyma. More-
over, the aerenchyma grew better in good light and the amount
of light necessary for good growth varied with the species. A
thorough understanding of the problem awaits experiments with
all these factors definitely controlled.

Gaseous Content of the Air Spaces

   A survey of investigations of the gas contained in the tissues of
vascular plants serves to emphasize three points, namely, the
ubiquity of gas-containing tissue, the high proportion of oxygen in
green tissue when exposed to light, and the positive correlation be-
tween carbon dioxide content of spaces and vital activity, in tissues
not engaged in photosynthesis.

   Even from succulent plants or from fleshy or woody organs, gas
for analysis can be obtained, and the results of analyses are in-
structive. In the air from inside sugar beets (55) the oxygen
content varied between 0.06% and 2.10% and the carbon dioxide
between 11.49% and 78.90%. In five species of succulents (2)
analyses of gas taken on a sunny day showed oxygen content of
18.35% to 26.45% and carbon dioxide content of 0.41% to 1.50%.
In apples, potatoes and carrots in storage (82) the percentage of
carbon dioxide in the intercellular atmosphere rose and that of
oxygen lowered significantly with increases in temperature, but

always the former was more plentiful and the latter less so than in
normal air. In gas extracted from the trunks of trees (79), the
carbon dioxide varied from 0% to 26% and the oxygen from 0%
to 22%. In branches and roots of grape vine and mulberry (37)
the oxygen was found to decrease and the carbon dioxide to increase
with increasing vegetative activity. When inactive the percentage
of carbon dioxide was very low, and the oxygen content approached
that of the air.

In an earlier section of this paper it was noted that gas pressure in
the floats of *Nereocystis* increased at times to points very con-
siderably above atmospheric pressure and that this increase was
contemporaneous with an increase in oxygen content. The same
has been found to be true in the intercellular spaces of aquatic
angiosperms, and gas from their lacunae will, in daytime, when
oxygen is being set free by photosynthesis, be forced out in bubbles
which can easily be collected. Van Tieghem (151) found such
gas, bubbling from wounds in *Elodea canadensis,* to be 90% oxygen.

GENERAL CONSIDERATIONS

The distribution of air-space tissue in the plant kingdom is inter-
esting. While one group of water plants, the algae, has less of this
tissue among its massive forms than any other, in a second group,
the aquatic angiosperms, air spaces reach their greatest development,
both in individual size and in combined comparative volume.

One cause of the scarcity of spaces in algae is undoubtedly the
character of the cell wall, particularly its primary layers, whose
tendency to swell with water into a gelatinous mass must impede or
often indeed preclude the development of the small schizogenous
spaces so characteristic of higher plants. This also would militate
against the development of large spaces of the schizogenous type if
the suggestion we have made should hold, namely, that such spaces
arise from special growth of the cells bordering the original small
ones.

Such wall peculiarity fails to account, however, for the scarcity of
lysigenous spaces amongst the algae. This, it seems to me, may
be accounted for by a physiological peculiarity of these primitively
aquatic plants. Diffusion of gases through water is very slow as
compared to that in air, and, as shown in an earlier section of this
paper, there is reason to believe that this, through its effect on res-

piration, causes enlargement of the lysigenous cavities in many higher plants. In the algae, however, we have to deal not with a group that has evolved for ages in air, with continuous ready access to oxygen, but with one whose cells have always been immersed and have a constitution that enables them to function normally under such conditions.

In the few cases where algae do produce spaces there are differences between them and the higher plants, and also similarities. In vascular plants, at first small, angular, liquid-filled spaces form from the pulling apart of cells. Normally they are soon united with each other and with the outside, and become filled with air. Whether this air comes from outside, or is secreted by surrounding cells, or owes its origin to both factors, there is still insufficient evidence to decide, though the first would seem unlikely in the case of submerged water plants. On the other hand, in vascular plants, the early shape of the spaces is not such as one would expect, if gases had been secreted into them under pressure. Moreover, in many Marchantiales, where the schizogenous spaces are reported to be in continuity with the outer atmosphere and full of gas from the first, the probability seems to be in favour of the entrance of outer air. In seaweeds, the air spaces, when present, do not connect with the outside. Careful studies of the earliest stages of their development seem rarely to have been made, but in *Fucus,* where such stages have been observed, they are said to appear as bubbles in the gelatinous walls, and it seems reasonable to assume that the gas responsible for their initiation and growth comes from the cells. The picture here, as obtained from evidence obviously still incomplete, is that of cells giving off gases into the interior of the frond and thus producing the bladder. In the Laminariales, even if the theory of Miss Kibbe with respect to *Alaria* should prove to hold for the group, and the spaces be found to be due to the tearing effect of differential growth, the gas with which they are filled can come only from the surrounding tissue. Miss Kibbe's hypothesis can not at present be accepted without reservation, however, for its explanation of some of the facts is not completely satisfying.

The large spaces which almost invariably develop in seaweeds where a beginning has been made as described above, may be compared to the large lacunae of the higher plants. In *Alaria* and *Lessonia* enlargement of the cavities was held by some to be due to

stretching and tearing of the tissues by rapid growth of the surrounding cells. In higher plants the same origin has been suggested for the lysigenous spaces, but subsequent work has thrown doubt on this theory, and a hypothesis of autolysis has been suggested. In so far as schizogenous lacunae are concerned, developmental study has been scarce except in the Marchantiales, where the original impetus arose from Leitgeb's view, considered erroneous by most modern workers, that no schizogeny occurred. In these chambers development is clearly bound up with a tendency to copious cell division on the part of cells in contact with the moist air of the space, and what we have seen of the form and development of schizogenous lacunae in vascular plants is indicative of the same tendency.

Some kinship between the lacunae in seaweeds and those in higher plants may be seen in two particulars. Three workers have reported in three genera, the disappearance or resorption of cells during the development of spaces, and in four, a stimulation of growth has been indicated, either by the growth of hairs or hyphae into the cavity, or by the development of an active cambium around it. It would thus seem that the fundamentals for the production of both lysigenous and schizogenous lacunae are present in some degree in the algae.

In Bryophyta the schizogenous type of air space predominates, though lysigenous spaces are found, particularly in parts of mosses not well supplied with chlorophyll.

The description by Martens and his school, of the first beginning of an air space, not by a mere splitting apart of cells but by inflation of the end of a newly-formed middle lamella after cell division, is interesting and seems reasonable in the light of our knowledge of young cells. If the chinks at the corners between cells come as a result of a rounding up process which in turn is due, as Priestley holds, to vacuolation and to elasticity of the primary wall, the break must come at the point where coherence of the intercellular substance is weakest, and it is to be expected that this spot will be found in the soft watery lamella that has just been laid down. We know that the middle lamella retains much of its softness for some time, and the fact that splits sometimes occur in the older side lamella in this same region of strain fits in very well with that fact. The occurrences at comparable points in the history of cell walls of Bryophyta and Thallophyta have yet to be investigated, and the problem may

prove difficult, for Martens found it possible to get a clear picture of what was taking place only in favourable material.

Whether the irregularly armed cells of the spongy mesophyll of leaves are physiologically akin to the star-shaped cells found in some mature lacunae and produced as-an ephemeral stage in the development of others, is a question that still awaits a definite answer. If they are, something must be assumed to happen in normal leaves to halt their progress along the course they have begun, a course that, followed to its conclusion, brings about their destruction. That happening might be the attainment of a more copious supply of oxygen or of food. Both would be available in greater quantity in leaves when photosynthesis reached normal proportions. Obviously other possible factors might be mentioned, but in the present highly theoretical stage of the problem, additional theorizing would not be profitable.

Before closing this paper some observations are in order on the place occupied by air spaces and lacunae in the plant's economy. Two possible spheres of usefulness present themselves as worthy of consideration, namely, aeration and, in the case of water plants, buoyancy and support.

In algae the aerating rôle of air-space tissue is of little consequence. In the bladders of *Nereocystis* it has been established (113) that oxygen accumulates in daytime when photosynthesis is active and disappears during the night, doubtless being used in respiration by the surrounding tissues. There is nothing to indicate, however, that these tissues are the better, either for this stored oxygen or for the supply of respiration products that accumulates during the night and disappears during photosynthesis. The tissue in the vicinity of the bladder has not been found to fare better than the much greater mass of cells constituting the rest of the plant or than the same tissue in youthful fronds without bladders. The ancestrally aquatic thallophytes are constitutionally in accord with an environment where diffusion is slow, and the additional retardation provided by the more or less swollen and gelatinous matrix of cell wall through which diffusion to and from the inner cells is free to take place, seems ordinarily to be harmless. The bladders do, however, serve as floats and supports for the massive, flexible, plant bodies.

Just as algae may be said to have their inner cells in direct communication with the water outside through the water imbibed in the

cell walls, so are the inner cells of land plants in contact with the outside air through the interconnecting intercellular spaces. Thus cells whose normal reactions require more rapid diffusion than is found in a liquid medium are able to function deep in the structure of the plant. The contention that this free and rapid transfer is important to such cells is upheld by the fact of their death in some cases when it is interfered with, as in McPherson's experiments, and also by the increased growth and cell division about the air spaces in plants that develop lacunae schizogenously.

Photosynthetic tissue in land plants, whether liverworts, mosses or vascular plants, is ordinarily provided with a particularly impressive system of air spaces, and the value of a free road for the diffusion of gases to and from the cells of such tissue will scarcely be denied.

Workers with aquatic angiosperms have given thought to the question of the benefit, if any, that these plants derive from the large lacunae so plentiful in their tissues. To the present writer it seems that the situation here is very different from that in seaweeds, and that these lacunae may be held to perform a useful function in providing for transfer of a supply of oxygen from photosynthetic cells to others. Diffusion of oxygen through water is much less rapid than that of carbon dioxide, a fact that has been held (1) to account for the increase of pressure in lacunae during photosynthesis. It has also been shown to be a factor in the death of cells of land plants under water, and remembering that aquatic angiosperms are descended from land plants, it is reasonable to assume that, since they are successful in their new habitat, this hazard must in some way have been eliminated. It is quite clear that, during the day, the concentration of oxygen in the lacunae of green plant parts becomes abnormally high. If this oxygen is to be transferred in any quantity to the lacunae of buried parts where, if anywhere, a special need for it would exist, the lacunae must form a continuous system. Barthélemy (4) gave evidence that this is so in *Nelumbium* and *Nymphaea*. He found not only that by blowing air into a cut petiole he could force it out through the stomata of the leaf blade, but also that by placing one leaf of an intact plant under reduced pressure in a belljar full of water, he could cause a continuous stream of air to enter the stomata of the free leaves, pass down through the tissues of the plant, and emerge into the belljar through

the stomata of the leaf enclosed therein. This experiment could probably be performed successfully with numbers of other water plants. It was done quite independently with *Sagittaria* some years ago, by an undergraduate in an ecology laboratory class at Toronto.

With a continuous system of lacunae from leaf to root, the diffusion of oxygen from the point of high concentration in the leaf toward the place of scarcity in the tissues without chlorophyll would seem inevitable. In addition there is the possibility of a certain amount of mass movement, the carbon dioxide of respiration from these tissues becoming dissolved and diffusing away, thus allowing room for an influx of oxygen-rich air from the upper lacunae where it is present under pressure. In plants with their leaves above water, a similar transfer of oxygen from the outer air through the lacunae might be expected during darkness. Something similar is also probable in the aerenchyma tissue whose development from cork cambium has been described, for this tissue extends to the surface of the water and often if not always to a point a little distance above.

It remains only to mention the importance of the buoyancy of the air-space tissue which largely compensates for the scarcity of mechanical tissue in submerged and floating angiosperms.

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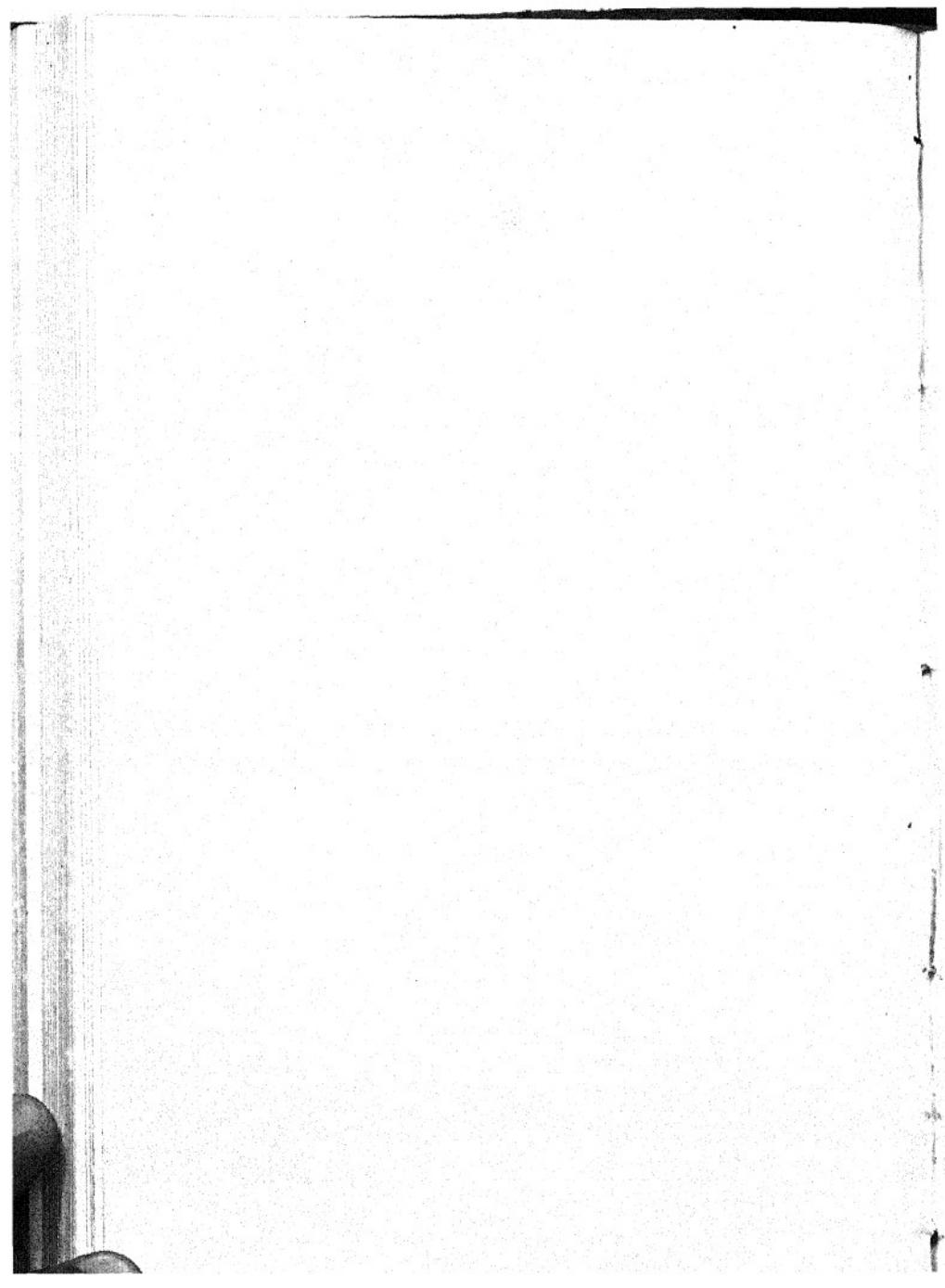
COLCHICINE AND X-RAYS IN THE TREATMENT OF PLANT AND ANIMAL OVERGROWTHS

### MICHAEL LEVINE
Montefiore Hospital, New York

INTRODUCTION

Colchicine, the alkaloid extracted from the seed and corm of *Colchicum autumnale* L., the meadow saffron, has been known for many years. Houdé, in 1887, isolated the substance as a yellow powder or flocculent soluble in cold water, alcohol, and chloroform. The drug found its way into the practice of medicine in the treatment of gout, yet its physiological activities in this disease have not been well defined. When injected into the body in appropriately small doses, it produces gastrointestinal symptoms, such as vomiting and diarrhea. In some respects it behaves like the alkaloid muscarine extracted from the fly mushroom, *Amanita muscaria*. Like pilocarpine, muscarine and physostigmine, colchicine excites nerve endings to glands and smooth muscles. In other respects it behaves like snake poison and toxins; it causes death after narcosis, during which reflexes disappear and respiratory failure follows. Lits and his associates (1938a) give a detailed account of the early uses of this alkaloid. Chemically, colchicine is composed of $C_{22}H_{25}NO_6$. According to Windhaus, the structural formula has a phenanthrene nucleus and is shown on the following page.

Dustin (1933, 1934) studied the action of arsenicals and colchicine on karyokinesis and cytokinesis in tumor tissue. These reports resulted in the development of a number of principal lines of experimental studies. Since Dustin's work, colchicine has been found effective in producing genetic changes in plants. The chromosome number in plants has been polyploidized and mutations have been produced at will. Colchicine has been used as a device to facilitate the study of the effects of hormones on various tissues of the animal body. It has been used as an aid in cytological studies, and has stimulated an interest in a search for other chemicals

which affect the normal processes in nuclear and cell divisions. Finally, this chemical coupled with x-ray irradiation has been used as a therapeutic agent in the treatment of cancer of man and animal, and bacterial overgrowths on plants.

These different phases of colchicine literature are reviewed separately and referred to below. The large number of reports now extant make it impractical to include all of them in a general survey. Here, only the salient features will be mentioned, and emphasis given to those studies which deal with the effects of this alkaloid alone or in combination with x-rays on animal and plant hyperplasias.
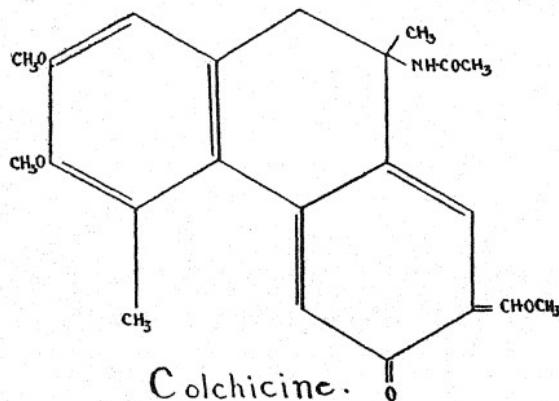
Colchicine.

The early studies on the effects of colchicine on cancer were fragmentary and without experimental basis. The belief that colchicine was an ameliorative in cancer seems to have been derived from some observations on gout patients who were concurrently suffering from cancer. In "Peau, Syphilis, Cancer" (1932) mention was made of Dominici's observations of cancer patients suffering from gout. He claimed these patients showed improvement and that the cancerous condition was arrested after treatment with colchicine. Dixon and Malden (1908) were the first to study the pharmacological properties of colchicine on the blood and bone marrow of rabbits, rats, dogs and on one or two cases of man. Rabbits injected subcutaneously with 1 cc. of 0.5% solution of colchicine caused transient leucopenia and a diminution in the polymorpho-

nucleate cell counts. Administration of large doses induced abnormal cells, and normoblasts were then commonly found. Small doses when repeated over several months produced no ill effects; the leucocyte counts remained unchanged. Treatments extending over long periods, however, produced definite changes in the bone marrow. The marrow cells increased and became more conspicuous, and fat cells disappeared. Very large doses of colchicine, the authors contended, produced destruction of all blood elements. Bone marrow elements were observed in the general circulation. Marrow smears showed an abundance of mitotic cell divisions. The significance of these observations was not apparent to Dixon and Malden.

The study of the mechanism of nuclear and cell division and attempts to alter their course have been in progress many years. Dustin had long been studying the action of various chemicals on cell division, and in 1933, in collaboration with Grégoire, he investigated the effects of sodium cacodylate and trypaflavine on mitosis of the well known mouse sarcoma, 180. Dustin (1934) continued these studies, using trypaflavine, rivanol, isamine blue, malachite green, arsenicals, benzol, iodine, zinc, mercury, thallium and certain microbic toxins which he described as "caryoclasiques" agents. Sodium cacodylate and typaflavine effects were studied on tumor grafts of the Crocker mouse sarcoma. The method employed by Dustin was subsequently used with modification by many investigators. It consisted of injecting small quantities of the chemical (30 mg. per 20 gm. of body weight) into the tumor grafts when they had attained the size of a cherry. The animals were sacrificed at intervals of 3 to 4 hours for the first day and at 48 hour intervals afterwards. Counts of the number of mitoses in the mouse sarcoma after sodium cacodylate injections, reached a maximum at 24 hours but showed a reduction at the 48th hour. Dustin (1934) believed that 0.025 mg. of colchicine had a stimulative effect on cell division in the sarcoma grafts much like that produced by sodium cacodylate. The number of nuclear divisions was calculated to be fifteen times greater than in the untreated tumor tissue. These divisions were followed by karyorrhexis. Colchicine, Dustin believed, produced the maximum number of division stages in the Crocker tumor at the ninth hour after injection. Similar reactions were observed in the Kupffer cells, reticulo-endothelial cells, the megocytes and testicular cells.

Dustin (1937) studied, further, the effects of numerous chemicals on cell divisions and believed the agents examined could be arranged into two classes, those that acted like trypaflavine and those that acted like arsenical compounds. The first class he characterized as one having essentially an inhibiting effect on karyokinetic division and resulting in "caryoclasic" shock. Substances in the cacodylate group, he found, produced reactions which he considered as violent excitants of mitotic division. He described the cytoplasm of these cells as turgescent, the prophase stages as of short duration followed by atypical metaphase stages. The prolonged metaphase stage, which became diagnostic for colchicine treatments, showed chromosomes markedly condensed, forming compact masses, which he described as "radiomimetic." These mitoses either disintegrated or completed the division of chromosomes without aid of a spindle structure. This division was frequently followed by development of "monstrous" nuclei. Dustin believed that malignant cells were highly sensitive to this alkaloid. Dustin (1938), too, showed that the dosage of colchicine required to bring about extreme mitotic activity in tissue of the mouse was a very small fraction of that required for the cacodylate. He found that reaction occurred principally in the generative zone or in cells at division induced by various conditions, such as trauma, inflammation, carcinogenic activity or hormones. Achromatic figures, asters and spindles were totally lacking in these colchicinized cells. While some cells showed complete pycnosis, normal telophase stages and cytoses occurred. Some cells assumed normal telophase stages and formed giant or polyploid nuclei. Dustin proposed the name of "stathmocinesis" for this type of indirect division and applied the name of "stathmocinetic poison" to colchicine. He contended that the arrest of the nuclear division in the metaphase was preceded by a phase of excitation which distinguished this poison from those which merely inhibited division.

Delcourt (1939a, b), working in Dustin's laboratory, studied the effects of colchicine (0.5 mg. to 250 mg.) at ordinary temperature on tissues of the lower vertebrates, frog, salamander and axolotl. He found that a pycnotic stage precedes excitation of mitosis. The dividing cells acted upon by colchicine showed a complete absence of spindle figures and the chromosomes were clumped as described by Dustin for tumor tissues. Delcourt found that the effects of

feeble doses of colchicine were augmented by higher temperature (37° C.). The maximum response in *Rana* to 0.5 mg. of colchicine occurred at 162 hours, while axolotl showed the maximum number of division stages at 183 hours after 0.025 mg. of colchicine.

COLCHICINE ON PLANTS

Havas (1937a), another of Dustin's collaborators, studied colchicine effects on plants inoculated with the plant tumor-producing organism, *Bacterium tumefaciens*. The stems of tomato plants inoculated with the bacteria were cut so as to free a spur of tissue by partial cross section near the base with longitudinal cuts extending for short distances up the stem. The spur on each plant was inserted on alternate days into a 1 : 10,000 solution of colchicine. Distilled water was used on the other days. Havas observed that colchicine stimulated the appearance of tumors, but when the end results were examined he found that the tomato stems treated with colchicine produced tumors that were but half as heavy as those of the controls. Havas' results led him to believe that colchicine was detrimental to the general development of the plant. Tomato crown galls painted with a mixture of lanolin and colchicine, in which the colchicine was 40 mg. per gram of the mixture, produced a reduction in the weight of the tumors as compared with those of the controls. Treatment by the spur immersion method of *Pelargonium* stems bearing tumors had no effect on the size of the tumor. Covering a scarred area of about 1 cm. on begonia stem with 3 mg. of a mixture of lanolin and colchicine stimulated development of the area, while similar treatment on *Impatiens* proved toxic in some cases though resistant specimens produced adventitious roots. Havas believed that colchicine acted as a local excitant of growth similar in action to that of a hormone. Colchicine, he contended, inhibited the development of plant tumors or crown gall on tomato. Havas (1937b) studied, further, the effects of colchicine on germinating seeds and seedlings of a pure line of Wilhelminia wheat. Roots and root hair development were stimulated at first, but the effects were followed by a depression in the growth rate and finally complete arrest. Colchicine effects were shown in these seedlings by the development of bulbous hypertrophy of the root tips and liquefaction of certain parenchymatous elements. Havas (1938) later attempted to show that colchicine effects were of a hormonal

nature, as evidenced by the appearance of adventitious roots,
"pseudo-neoplasms" on root tips, hypertrophy of the coleoptile in
seedlings, and stimulation of somatic growth of *Begonia*. Tomato
plants showed epinasty when the leaves were stroked with the finger
dipped in a 5:1,000 solution of colchicine. The response developed
in 40 minutes and lasted four to six hours. *Tradescantia* showed a
hyponastic reaction when sprayed with a 1:20,000 solution of the
drug. Wounds on cherry laurel leaves healed in a few hours when
treated with 1:2,000 to 2:1,000 colchicine solution. Havas be-
lieved he was dealing with a "phytohormone" comparable with the
wound hormone, traumatin. Yeast was stimulated to growth by
colchicine and thus behaved like bios or vitamin B, according to
Havas. Yet he concluded that colchicine was not a hormone but a
mobilizer of the hormone already in the plants. The hypertrophies
produced on root tips Havas (1939) believed analogous to the
crown gall disease.

The contention of Dustin and his associates that colchicine has a
therapeutic value in tumor growth resulted in many studies designed
especially to determine this point. Animal pathologists directed
their attention to the effects of colchicine on a number of differ-
ent types of neoplasia in animal and man. The plant disease known
as crown gall first tested with colchicine by Havas was also studied
intensively in this connection. So far, no other type of plant over-
growth commonly referred to by botanists as plant tumors, such as
the potato wart, club root and nematode galls, has been investigated
in its relation to colchicine. Brown (1939), who was one of the
first investigators associated with experimental production of crown
gall, made a series of investigations to determine the therapeutic
effects of colchicine on these bacterial overgrowths. She attempted
to prevent crown gall formation as well as to destroy it when fully
formed. Eight species of plants were under observation, Paris
daisy, French marigold, four o'clock, *Bryophyllum, Kalanchoë,
Nicotiana glauca,* kidney bean and tomato. Plants treated with
colchicine by the Havas method failed to prevent formation of crown
gall. A delay in tumor formation was observed when the concen-
tration of the colchicine solution used was 0.5% to 2%. In some
cases the tumors formed were smaller than in the controls; in other
trials the colchicine injured the plants severely. Those plants
which were inoculated with the bacteria and then injected with

0.2 cc. to 0.5 cc. of a 2% solution of colchicine produced nine tumors in 61 individuals of five species of plants. Colchicine dwarfed tomato and marigold plants, although these effects appeared to be temporary in the tomato. The areas inoculated with *B. tumefaciens* were covered with lanolin to which 3% colchicine was added. Brown found that this treatment delayed tumor formation but that the tumors grew to more than half the size of the controls. Overgrowths produced by indoleacetic acid when similarly treated with colchicine produced like results. Brown concluded that colchicine did not prevent the formation of overgrowths after chemical or bacterial treatment.

In an attempt to kill or prevent further development of fully grown crown galls, a colchicine solution was painted on the surface of these growths or a lanolin colchicine paste was applied. Growth of crown gall on Paris daisy was inhibited and the tissue blackened in 16 days; at the end of 70 days the galls were dead, although the stems above and below the overgrowths were alive. A more interesting observation made by Brown concerned the failure of 0.5% solution of colchicine to cause death of tumors on the tomato plant, although a 2% solution interfered with the growth of the galls so that controls were twice the size of the treated ones. *Kalanchoë* stems bearing crown galls set in 0.5% solution of colchicine for four hours and then transferred to water for ten days produced roots at the galls, while controls showed no roots; the treated tumors blackened and died.

Brown believed that death of the galls was not due to direct killing or inhibiting actions of colchicine on the bacterial organisms, but, in accord with Havas, contended that the colchicine affected the growth substances in the plant.

Brown (1942) further studied the effects of acenaphthene, methylnaphthalene, α-nitronaphthalene, 3,5-dibromopyridine, heptyl aldehyde and apiole. These substances were incorporated in a paste made of lanolin. Apiole induced dwarfing of the treated plant but no blackening of the tissue nor death; α-methylnaphthalene (50% and 100%) inhibited growth; heptyl aldehyde, as well as α-methysalicylate, used in full strength caused death. The age of the tumor, Brown pointed out, affected the survival rate of the tumors; young tumors were inhibited, old tumors were killed. Dermen and Brown (1940a, b) studied the cytological effects of

colchicine on plant tumors. Twenty day-old tumors on African marigold and French marigold were used, to which various solutions of colchicine varying from 0.025% to 1% were painted on the surface of the gall. The treated tissues were removed at intervals of 2, 5, 7 and 9 days after treatment. The cellular changes found were associated with excessive polyploidy and multiploidy in the affected meristematic cells of the tumor tissue. The effects induced by the colchicine were not immediate for growth in controls, and treated tumors continued for a week. Then the treated tumors blackened and eventually died. The principal cytological change which accompanied these morphological ones was multiploidy. This condition resulted in large nuclei in hypertrophied cells of the treated tissue. The size of the cells so produced had definite limits, the authors believed, beyond which death followed. Dermen and Brown suggested repeated colchicine injections in conjunction with irradiation as a therapeutic measure for cancer.

Solacolú, Constantinesco and Constantinesco (1939) treated crown galls on stems of *Pelargonium zonale* and *Ricinus communis* with colchicine. The galls were covered with a paste of lanolin hydrate in which 0.5% to 0.75% colchicine was introduced. This treatment was repeated three or four times at 15-day intervals, and as a control lanolin was used alone. These authors observed a layer of meristem tissue formed between the tumor and the host. This new tissue became suberized and thus cut the metabolic exchange between the plant and the tumor so as to kill the new growth.

COLCHICINE ON ANIMALS

Lits (1936), another of Dustin's associates, investigated the cellular lesions in animals produced by this alkaloid. Injection of rat tumor with the chemical frequently resulted in the production of cells too abnormal to be viable. Aberrant cells frequently met in malignant growth were destroyed. Lits believed that the cytological effects of the colchicine resembled those induced by radium. He contended that colchicine was more "caryoclasique" than "caryocinetogenic". Rat tumors, benign or malignant, were found sensitive to the radiomimetic-inducing properties of colchicine. Lits, Kirschbaum and Strong (1938b) emphasized the radiomimetic properties of colchicine attributed to it by Dustin, and studied its effect on lymphoid tumors of the mouse. Mice of the Strong C_8H

strain were injected with leukemic lymphoid tissue, and when the transplants had attained the size of a kidney bean (14 days), 1/40 mg. of colchicine in distilled water was injected subcutaneously. This injection was repeated at intervals of three days. Complete disappearance of the local growth occurred. Survival time of controls after the transplantation was 31.5 days as compared to 50.5 days in colchicine-treated tumors. In no case was there complete regression without recurrence. Histological examination of the tissue after the third injection showed all lymphoid cells of the tumor in pycnotic condition or dead; the reticular cells of the lymphosarcomatous growth remained. Colchicine was found to hasten death in those animals where the lymphoid neoplastic disease was systematic.

Amoroso (1935), observing a beneficial effect of colchicine on gout patients who had been treated for cancer with x-rays, tested the efficacy of colchicine on cancerous growths (Carcinoma M63) on white mice. The tumors on these mice grew slower than the controls, and at the end of two weeks there was no microscopic evidence of them in two thirds of the tumor animals. The remaining third showed complete regression eight weeks later. In another series of similar mouse tumor experiments there was no recognizable tumor development in two weeks after colchicine injections were made. In a spontaneous tumor in the peritonsillar region of a dog, Amoroso recorded complete regression after injection with colchicine. The combined effects of x-rays and colchicine were not studied. Poulsson (1935) used 15 mice with malignant tumors, five spontaneous mammary carcinomas and ten with tar cancer to determine the curative effects of colchicine. He injected 1/80 mg. of colchicine subtucaneously and found that the drug had no effect on development of the tumors. The tumors ran their usual course terminating the life of the animal by ulceration of the tumor with infection and hemorrhages.

Peyron, Lafay and Kobozieff (1936) tested the action of colchicine on the Shope rabbit papilloma. The rabbits were injected eight or nine times over a period of three to four weeks with an average dose of 1.5 mg. of colchicine. The authors reported complete regression of the tumors without recurrence. In a later paper (1937a), in collaboration with Poumeau-Delille, Peyron and Lafay injected rabbits with Shope papilloma with slightly smaller doses

(1 mg.) of colchicine. The rabbits carried tumors of different ages varying from one to four months. The control rabbits, with the exception of the cases of spontaneous regression, went through the usual changes characteristic of this disease followed by death. The colchicine-treated animals were free from the tumors within three to five weeks. In two series of experiments, colchicine was applied in aqueous solution of 1/100 or in paste in concentrations of 1/20, 1/50 and 1/100. In another series the papilloma was introduced into both flanks of the animals. Only the tumor treated with colchicine showed rapid and uniform regression. The untreated tumors became stationary and regression occurred at a much slower rate. Of 19 animals, eight died before the regression of the treated side was completed. These deaths were associated with secondary infection, intoxication and ulceration. In the 11 survivors, regression of the treated tumors was completed or nearly so; six to eight weeks sufficed if the treatment with colchicine was continued. The authors expressed the opinion that colchicine given orally or by local applications might be beneficial in precancerous lesions or benign tumors in man.

Clearkin (1937) studied the effects of subcutaneous injections of colchicine on transplants of mouse sarcoma 37. The animals inoculated with tumor fragments were injected with 0.0015 to 0.003 mg. of colchicine per 20 gm. of body weight. This was followed on alternate days by injection of 0.01 mg. of the alkaloid. No significant difference was noted between these animals and the tumor controls. Mice bearing tumors approximately 1 cm. in diameter were injected with 0.01 mg. of colchicine every other day. In this series no difference was observed between the tumor-bearing animals treated and those not treated. The rate of growth was the same in both groups. Mice were inoculated with bits of tumor from a mouse which had received 0.01 mg. of colchicine; three days later they were injected intravenously with 0.04 mg. of colchicine. One hour after the injection, the transplanted tumor tissue was removed and implanted into fresh hosts. Controls were similarly treated except for omission of the colchicine injection. The tumor tissues from both sets of animals were also cultured *in vitro*. There was no difference in the number of takes of the experimental and control series *in vivo*. There was, however, a slight retardation in the rate of growth of the tumors derived from the colchicine-treated

animals during the first week after their appearance. The end results, however, were similar. Cultures *in vitro*, however, showed that 60% of the colchicinized tumor transplants failed to grow. In the remaining cultures growth was very slight. The controls, *in vitro*, showed vigorous growth in 90% of the cultures. Clearkin believed that sarcoma 37 was less affected by colchicine than many normal tissues.

Ludford (1936) was among the first to study the effects of colchicine on animal tissues. He studied the effects of this and of other chemicals, such as auramine, urethane, methylsulphonal and sodium cacodylate, on mouse carcinoma 63 grown *in vivo* and *in vitro*. Tissue cultures of this tumor grown in a medium to which colchicine in varying concentrations of 1 : 100,000 to 1 : 100 million were investigated. Solution 1 : 100,000 to 1 : 800 inhibited outgrowth of the transplant and arrested cell division. In those cultures in which mitoses were brought to a standstill, the nuclei of the resting cells were appreciably altered. Concentrations of colchicine which inhibited mitoses also affected resting nuclei of normal and malignant tissue. Ludford's experiments showed that the reactions of cells to colchicine or sodium cacodylate were essentially alike but that the former induced reaction at a much lower concentration than that required by the latter. Colchicine, Ludford showed, was active over a wider range of concentrations. Ludford is credited with having pointed out the inhibiting action of colchicine; the stimulative effect at first claimed by Dustin and his coworkers were shown to be an accumulation of the arrested cells in metaphase stage. Schairer (1940) used ascitic cancer of the mouse *in vivo* as the test material. Besides observing the typical colchicine phenomena Schairer noted that the mitotic changes appeared in two hours and the accumulated mitotic figures became prominent at 15 hours. Like Ludford (1936) this author concluded that a curative effect with colchicine could not be attained without injury to the animal.

Tennant and Liebow (1940) also resorted to tissue culture for testing the effects of colchicine and ethylcarbylamine. They believed that by this method the concentration of the chemicals actually in contact with the cells was known. The tissue used consisted of mammary cancer in mice induced by theelin and breast tissue of new-born mice. Colchicine, they found, reduced the ex-

pansion rate of the colonies of the mouse breast cancer in concentration as low as 1:64 millions. The rate of mitosis was reduced but the cells in mitosis accumulated. Many cells remained alive, as demonstrated by successful transplants. Ethylcarbylamine acted like colchicine but its activity was confined to a narrower but higher range of concentrations. Bucher (1939) studied the growth rhythms of fibroblasts in tissue cultures. He added trypaflavine or colchicine to cultures of these cells and compared their reactions. Colchicine, Bucher held, retarded the division rhythm with initial injury in two to three hours. Trypaflavine blocked more or less completely the beginning of new mitoses. Bucher believed that the disturbance produced by colchicine or trypaflavine is one of a regulative mechanism.

Gavrilov and von Bistram (1939) studied heart and iris tissue of the chick *in vitro*. Colchicine in low concentrations acted as a specific mitotic poison producing vacuolization and granulation of the cytoplasm. A new form of cell induced by colchicine was described as "monocytoid" which was characterized by a compact and deeply stained nucleus. These cells were derived from macrophages and fibroblasts. When the action of colchicine was not too severe, the "monocytoid" cells recovered and regenerated into macrophages and fibroblasts.

Brues (1936) investigated the effects of colchicine on the regenerating liver of the rat following partial hepatectomy, as a prelude to his studies of the therapeutic value of this drug on cancer. The animals were injected with 0.1 mg. or 0.2 mg. of colchicine per 100 gm. of body weight 22 hours after operation, and then sacrificed at eight hour intervals. Microscopic examination of the livers failed to show a stimulating effect of the alkaloid, as contended by Dustin, although large doses induced abnormal mitoses. Brues and Cohen (1936) studied the effects of colchicine and colchicine derivatives on cell division. They found that a comparable dose of sodium cacodylate gave essentially the same cellular responses as colchicine. Partially hepatectomized rats were injected with an aqueous or oil solution of colchicine varying in strength from 0.1 mg. to 1 mg. per 100 gm. of body weight of the rat. With the smallest doses the number of arrested mitoses was small, and while most of the chromosomes were clumped, some cells showed scattering of chromosomes. With injections of 0.2 mg. of colchi-

cine, optimum results were obtained for the number of arrested mitoses. The largest dose (1 mg.) caused death coupled with the smallest number of mitoses. All the colchicine derivatives used (octahydro-colchicine, N-acetylocolchicine and 4 derivatives of the latter compound) produced effects similar to those of colchicine, but only when applied in considerably higher doses. Dimethyl and trimethyl colchicine acid were found effective in arresting mitoses in any sublethal or lethal dose. Scheifley and Higgins (1940) also studied the effects of partial hepatectomy on male white rats in which 70% of the liver was removed. The toxic effect of the colchicine as measured by mortality was found to be inversely proportional to the amount of the liver present.

COLCHICINE AND X-RAYS

Brues and Jackson (1937) studied the nuclear abnormalities resulting from inhibition of mitosis induced by colchicine and other substances. Rat sarcoma was studied ten hours after an injection of 0.025 mg. of colchicine. The peripheral cells in the tumor showed the greatest number of mitotic figures. Brues and Jackson believed that the size of the dose determined the degree of aberrant cytological behavior. In a later paper, Brues, Marble and Jackson (1940) studied the effects of daily treatments of colchicine on animal tumors and regenerating tissue. They believed that a narrow range of dosage existed which caused the characteristic effect upon cell division and that this dosage could be injected daily without causing death of the animal. The belief that cells in mitosis were most susceptible to the effects of Roentgen radiation led them to study the influence of x-rays on colchicinized tissue. Radical operations were performed in an effort to affect recurrences and metastases from small nests of cells which the alkaloid might readily penetrate. Four groups of experiments were arranged; a control, a group in which only colchicine was injected, a third group in which the tumor was irradiated and a group in which the combined treatments of colchicine and x-irradiations were administered. The exposure to x-rays were made in ten daily treatments usually of 300r each. The x-rays were given 16 hours after the colchicine was injected. The results obtained in the colchicine series alone showed no regression but the tumors remained quiescent or retarded. No satisfactory statistical analysis of growth rate differences between this

group and the controls could be made. In the study of the combined effects of colchicine and irradiation the results were different in two mouse tumors, C67 and 180. Tumor 180 showed only a slight regression after 3000r. The mouse tumor ceased to grow four to five days after beginning of irradiation and assumed its usual growth three to ten days after the irradiation was discontinued. In the Walker rat tumor, five tumors of 24 regressed permanently and one disappeared temporarily. Rat tumors ceased to grow on the second to fifth day after beginning of irradiation and remained quiescent for seven to ten days, after which they disappeared rapidly or resumed growth slowly. There was little difference between the tissues irradiated only and the tissues given the combined colchicine and irradiation treatment. Brues and his associates believed that colchicine had no effect on the response of these tumors to irradiation. Regression began no sooner after irradiation where colchicine was given than where it was not given. Colchicine did not even decrease the latent period between irradiation and various stages of regression as might be expected of regression dependent upon destruction of cells which were in mitoses at the time the irradiation was given.

Guyer and Claus (1939) used 672 rats with Flexner-Jobling carcinoma in a series of experiments to test the combined effects of colchicine and x-rays on this tissue. They found that 0.1 mg. of colchicine per 100 gm. of body weight administered subcutaneously coupled with irradiation of 3,000r proved a lethal dose to these animals in two to three weeks; 4,500r given in three doses of 1,500r each administered at intervals of two weeks or more were better tolerated, but smaller doses of 188r to 375r, frequently repeated, proved more effective than the larger doses. They counted the number of divisions in the tissue at various periods after the injection of colchicine. They found that rat tumor tissue prior to colchicine injection showed an average of 6.3% mitotic figures in 3,000 cells counted, while 15 hours after the colchicine injection the mitoses increased to 38.2%, which represented the highest number obtained. In another experiment the tissues from colchicinized tumor-bearing rats were removed, cut into equally thin slices and exposed simultaneously to 1,500r. This tissue was then transplanted into normal rats. All of the 89 rats classified as nontakes were later implanted with non-treated tumor grafts; 63 of

these animals developed tumors. Guyer and Claus believed they showed that far greater lethal effect was produced by the combined effects of colchicine and x-rays on cancer tissue than by x-ray treatment alone or of colchicine alone. In studying the combined effects of colchicine and x-rays on living rats bearing tumors, 91 animals were used. These were divided into three groups; 32 were treated with colchicine and x-rays, 29 with x-rays, and 30 with colchicine. The x-rays were given in small doses of 188r twice weekly. The x-ray treatments were started 16 days after the implanted tumor attained the size of 1 cm. to 1.5 cm. and 15 hours after the colchicine was injected. At the end of six weeks, the group which received both x-rays and colchicine showed a large number of animals healed and few deaths were recorded. Guyer and Claus concluded that x-rays were markedly more lethal to bits of cancer tissue from colchicine-treated animals than to comparable pieces of cancer of untreated animals, as shown by infrequency of takes on transplantation to susceptible animals. Administering colchicine to rats bearing actively growing tumors 15 to 18 hours before irradiation, x-rays were found effective in completely destroying or in retarding the tumor growth.

In a later paper, Guyer and Claus (1940) studied the effects of injected distilled water on Flexner-Jobling rat carcinoma 15 hours after subcutaneous injection of colchicine. The water was injected into the tumors. After one week the surface of the tumors sloughed off and many of the growths appeared to be healing. Repeated injections of distilled water were made at intervals of two weeks. In four series of experiments 103 rats were used and healing was observed in 59 cases (57.3%); 18 tumors remained unhealed; 26 animals died.

Oughterson, Tennant and Hirschfeld (1937) reported the effects of subcutaneous or intramuscular injections of colchicine on 21 cancer patients. Of this number, 15 permitted biopsies before the alkaloid was administered. After the colchicine was injected biopsy material was again taken or the entire tumor was removed nine and a half hours after the injection. The doses of colchicine varied from 1 mg. to 4 mg., and as little as $1\frac{1}{2}$ mg. produced the cellular changes typical of colchicine effects. In some cases the authors were able to secure frequent biopsies which enabled them to count 19.6 cells in metaphase stage per high power field 12 hours

after the administration of colchicine as compared to 2.6 mitoses in
the controls. Oughterson, Tennant and Laurence (1940) studied
the effects of x-rays on a transplantable mammary carcinoma in
strain A mice. Irradiating with 5,000r, cures were effected in
48% of the mice, while hybrid mice showed cures with 2,500r and
5,000r, respectively, varying from 82% to 97%. Tumors that
responded best to x-ray therapy, they believed, developed a charac-
teristic stroma. They considered the host an important factor in
determining the response to irradiation. In a later paper, Hirsch-
feld, Tennant and Oughterson (1940) studied the combined influ-
ence of colchicine and x-rays on a strain of mice with transplantable
mammary carcinoma and a breast tumor of a male mouse resulting
from injections of oestrone. The mice were treated when the tumor
transplants attained 0.5 cm. to 0.7 cm. The colchicine dose was
calculated in milligrams per gram of body weight and administered
in 5 cc. of saline. The effects of various doses of the alkaloid were
studied. Attempts to cause regression of the tumors with single
or repeated doses of the drug were also made. The colchicine had
no effect on regression of the spontaneous tumors of strain A mice.
The combined effects of colchicine and x-rays on the growth of the
transplantable mammary tumors were studied. The authors con-
tended that a single dose of 0.0008 mg. of colchicine per gram of
body weight produced contracted metaphases which appeared most
abundant at four, five and six hours after injection. When the
animals were injected with 0.0007 mg. per gram of body weight
and this dose was repeated nine times at intervals of nine hours, a
marked increase in mitotic figures was observed at the 27- and 36-
hour intervals. Necrosis appeared at 36 hours; at 81 hours all the
animals died. Injection of 0.0016 mg. of colchicine at three- to
five-day intervals caused retardation of growth and prolongation
of life of the tumor animals. Animals with mammary tumors 0.4
cm. to 0.5 cm. in diameter were irradiated eight to ten hours after
each was given a single subcutaneous injection of 0.0016 mg. or
0.001 mg. of colchicine per gram of body weight. Mice were irra-
diated eight to ten hours after a second dose of colchicine was ad-
ministered at an interval of nine and a half hours. In these experi-
ments a dose of colchicine which produced a maximum accumula-
tion of mitoses plus a single exposure to irradiation with 2,500r
gave a slightly higher ratio of curability than x-rays alone. How-

ever, these authors believed the results insufficiently significant to warrant further study. Colchicine combined with 5,000r gave results similar to those obtained with x-rays alone. Colchicine, these authors concluded, did not strikingly increase the destructive effects of x-rays upon the tumor.

Seed, Slaughter and Limarzi (1940) studied the effects of colchicine combined with x-rays on advanced carcinomas of man. The four cases reported consisted of two breast adenocarcinomas, one rectal tumor, and one unclassified mass in the neck of a male. The colchicine dose given was repeated at intervals of seven days following x-ray irradiation. Two of the subjects died of colchicine poisoning. Two patients were irradiated, one of whom was treated before the colchicine was administered. Biopsies made after 10 mg. of colchicine were administered showed numerous mitotic figures in metaphase. The tumors necrotized, but after considerable sloughing, growth proceeded rapidly and death followed.

COLCHICINE AND BACTERIAL FILTRATES

Shwartzman (1936a, b) described the production of hemorrhage and necrosis of tumors by injection of bacterial filtrates into animals. Andervont (1940) studied the effects of colchicine and of bacterial products on animal tumors. He also tested the effects of repeated injections of colchicine on the growth of spontaneous mammary tissues and the combined effects of colchicine and bacterial products. Colchicine in large doses (0.15 mg. to 0.2 mg.) caused hemorrhage but also proved lethal, while smaller doses caused correspondingly less hemorrhage without much change in the tumor. Filtrates of *B. coli* passed through an N Berkefeld filter showed wide range of hemorrhage potency. Andervont concluded that colchicine and bacterial filtrates supplemented each other in producing hemorrhage and regression of transplantable mouse tumors, but the combined effects were no greater than that of a single injection of colchicine. Ascorbic acid which prevents tumor hemorrhage after bacterial filtrate injections proved ineffective after colchicine.

Boyland and Boyland (1937–1940) showed that tumor-bearing mice and rats injected with a near lethal dose of colchicine produced hemorrhage in the tumor with a reduction in the ascorbic acid content of the tumor, also a reduction in metabolism. They believed

that filtrates of *B. typhosus* and colchicine resembled each other in
the effects they produced. They found that a given dose of col-
chicine was more toxic to tumor-bearing mice than to normal mice
and that ascorbic acid had no favorable effect on the survival rate
of tumor mice. Studies were made on transplanted, induced and
spontaneous mouse tumors after intraperitoneal injections of col-
chicine followed by x-rays 18 to 20 hours afterward. Respiration
and anaerobic glycolysis were measured and the vitamine C con-
tent was determined. The degree of hemorrhage was estimated
macroscopically. The authors concluded that the spontaneous
tumors appeared much less affected than the grafted tumors and
somewhat less than the methylcholanthrene-induced tumors. Boy-
land and Boyland were the first to observe that ascorbic acid did
not neutralize the hemorrhage-producing power of colchicine. They
observed that 2-methylnaphthoquinone did not alter the effects
produced by colchicine.

ACENAPHTHENE AND OTHER CHEMICALS

The effects of trypaflavine, an arsenical compound, and of col-
chicine on mitoses in pathological and normal animal and plant
tissues stimulated interest in search of other chemicals which would
induce similar changes. Dustin, Lits, Brues, Brown and others
have studied a number of chemicals with this point in view.
Schmuk (1938) and later in association with Gusseva (1939)
showed that acenaphthene, like colchicine, could induce cells with
multiples of the species-number of chromosomes. The assumption
that a certain relationship existed between these substances and the
carcinogenes led Schmuk and Gusseva to test many animal tumor-
producing substances for their ability to induce polyploidy. The
chemicals were dissolved in ether and poured over the filter paper.
When the ether evaporated, the paper was moistened with water,
and seeds of corn, wheat and barley were placed on it to germinate.
Schmuk found that wheat and barley seeds were polyploidized by
acenaphthene, acenaphthylene, chloracenaphthene, bromacenaph-
thene, α-chlornaphthalin, α-bromnaphthalin, α-iodonaphthalin,
α-dibromobenzol, methylnaphthalaether, 3,5-dibrompyridin, ethyl-
naphthoat and α-nitronaphthalin. Germinating legume seeds did
not become polyploid from but were resistant to the effects of ace-
naphthene. Kostoff (1938a) followed Schmuk with studies on

germinating seeds of wheat, rye, oats, barley, maize, rice, *Festuca* and *Lolium*. The seeds were covered with crystals of this compound for four to eight days and allowed to grow slowly. Legumes (*Vicia, Erodium, Lothyrus, Medicago*) reacted slowly after an exposure of six to twelve days. Compositae responded in four to eight days. Kostoff showed that mitoses proceeded abnormally, no spindle fibers were produced, no equatorial plate stages appeared; the chromosomes divided but did not move to the poles, thus forming polyploid nuclei. The chromosomes were spread in the cytoplasm, frequently giving rise to polynucleate cells. Floral buds of nine species of *Nicotiana* were treated with crystals of acenaphthene. Abnormal microspores and abortive pollen grains were found. The viable pollen grains had unequal numbers of chromosomes. Kostoff (1938b) induced chromosome doubling in species of *Triticum* and *Secale* by subjecting the seeds to a saturated solution of acenaphthene with excess of crystals. Acenaphthene, Kostoff stated, acted like colchicine by creating conditions for polyploidy in *Triticum, Nicotiana* and *Lactuca*. He pointed out that long exposures to large amounts of sublimated particles of acenaphthene may injure or even kill plant tissues. Nebel's (1938) results with saturated solutions of acenaphthene on *Tradescantia* gave no polyploidy. Acenaphthene, it must be remembered, is only very slightly soluble in water. The sublimated crystals, it seemed, alone produced reactions. Kostoff (1938c) later concluded that colchicine was toxic, while acenaphthene did not appear to be. In a later report, Kostoff (1939d) induced polyploidy in some series of cereals by subjecting them to pulp from disintegrating corms of *Colchicum autumnale* L., which suggested the possibility of finding polyploid plants among the species growing in nature in association with *Colchicum*. Bates (1939) tested this theory and found that other plants growing among corms of the meadow saffron revealed no abnormalities. In nature, Bates believed the polyploid individuals were suppressed in competition with the normal seedlings or established vegetables.

Gavaudan, Gavaudan and Durand (1938) tested acenaphthene on germinating wheat seedlings. They found themselves in accord with Kostoff in that acenaphthene prevented spindle formation. These investigators also tested the efficacy of diphenyle and naphthalene and their derivatives on dividing cells. While arrested mitoses were observed, some of the derivatives like diphenylmethene

were toxic. Gavaudan (1941) tested (1,2,3,4,5-tetramethylben-
zene) durene and found that it induced mitotic inhibition similar to
acenaphthene. Gavaudan and Gavaudan (1939b) discovered that
apiole, an extract of parsley, inhibited cell division. They found
that *Hordeum* seedlings were very susceptible to apiole and showed
after the first 24 hours tetraploid and octoploid cells. Karyokinetic
anomalies were varied in the species studied. After colchicine, the
authors believed, apiole was the second known substance existing
in a natural state capable of modifying nuclear and cell division and
eventually producing polyploidy.

Simonet and Guinochet (1939a) reported the production of poly-
ploidy in flax seedlings with paradichlorobenzene. This substance,
they believe, produced morphological changes similar to those ef-
fected by colchicine and acenaphthene. Simonet and Igolen (1940)
investigated the effects of the vapor of the oil of leaves of *Citrus
nobilis* on flax and on barley seedlings. Roots of the latter were
more sensitive than flax. Marked disturbance of cell and nuclear
divisions were observed. Anaphase and telophase stages were
lacking and the cells became multinucleate. The principal constitu-
ent of the oil, methylester of methylanthranilic acid, produced the
same reactions. Simonet and Guinochet (1939b) obtained effects
with α-chloronaphthalene and α-bromonaphthalene, which they be-
lieved comparable to those induced by colchicine. Simonet (1940)
also claimed to have produced colchicine-like anomalies with 1,3,5-
trinitro-m-xylene when barley and flax seedlings were tested.
Shimamura (1939b) used sublimated acenaphthene on young flower
buds of *Fritillaria* and observed abnormal nuclear division in pollen
mother cells. The pollen mother cells divided into many cells, each
containing small nuclei. Hukusima (1939) reported the produc-
tion of polyploids in *Brassica alboglabra* with the aid of acenaph-
thene. The flowers were encased in small glass tubes, the walls of
which were covered with crystals of acenaphthene. Favorski
(1939) listed 12 chemicals among which were aurantia, tribromo-
aniline, α- and β-naphthylamine, trinitrophenol and tribromophenol.
These substances affected mitoses analogous to colchicine. Garri-
gues (1939) compared the effects of chloral and colchicine on the
roots of *Vicia faba*. Chloral, he reported, did not induce swellings
of the root; it acted on the resting nucleus, changing its structure,
and affected cells above the meristem, thus interfering with the

cells in the elongation zone. Mol (1939) applied acenaphthene in paste form mixed with formalin, alcohol or water to roots of gladiolus. The colchicine-formalin paste killed the roots, while the alcohol mixture injured them. Mol concluded that acenaphthene is less effective than colchicine. Weichsel (1940) used auramin on *Vicia faba* and barley and acenaphthene on *Soya*, luzerne and corn. She reported changes similar to those obtained by Schmuk and Kostoff on the grass seedlings with bromnaphthalene and bromacenaphthalene. Fatalizade (1939), too, showed that crystals of acenaphthene acted on F_1 seedlings of a cross between *N. rustica* × *N. paniculata* and produced polyploids.

COLCHICINE IN GENETICS

Application of colchicine in the study of plant genetics has been productive of new types of plants, the merits of which must still be determined. Blakeslee (1939), who is the leader in this phase of colchicine work, believes that through the application of this alkaloid, new species of plants may be created at will. Blakeslee and Avery (1937) described the various methods of applying colchicine to organs and tissues of the plant. A long series of papers on the effects of colchicine on economic plants has been published. Judicious use of the agent in plant genetic studies has opened a productive field of scientific endeavor and has added a new feature to colchicine little suspected by the early writers. It would be beyond the scope of the present report to review these publications. Attention is directed to a small number of these papers which tend to summarize this vast though new literature and to give some of the general results obtained with colchicine. Emsweller and Ruttle (1941) summarized the use of colchicine in floriculture. Badenhuizen (1941) described the induction of tetraploid economic plants such as tobacco, jute and hemp. Sell (1939) applied colchicine to seedlings of pasture plants such as alfalfa, bur clover, vetches, with satisfactory survival of seedlings. Thompson and Kosar (1939) induced polyploidy in lettuce. Shimamura (1938) produced fertile tetraploid tomatoes. Becker (1938) studied the influence of colchicine on some common vegetables, viz., beans, onions, peas, potatoes, rhubarb and asparagus. Weller (1940) effected chromosome changes in sugar cane seedlings; seeds treated with colchicine showed higher percentage of germination and growth stimulation.

Mendes (1940) described octoploid and tetraploid cotton plants derived from colchicine-treated seeds. Production of tetraploid rubber-producing dandelion (*Taraxacum Kok-saghyz*) was reported by Navashin and Gerasimova (1941). The use of colchicine in fruit breeding was the subject of another report by Nebel and Ruttle (1938b).

COLCHICINE IN CYTOLOGY

Many of the studies on colchicine reported above were accompanied by cytological studies of germ plasm as well as somatic cells, and many cytological investigations *per se* have been made after use of the drug.

Dermen (1940) reviewed the literature on polyploidy as induced by colchicine and has given an adequate summary of the technique employed in the production of these aberrant chromosomal behaviors in plants and animals. Wellensiek (1939) has given a short digest of the use of colchicine in polyploidy as well as an historical account of the properties of the alkaloid. Györffy (1940a, b) studied the chromosome numbers of polyploids obtained by colchicine treatment, and has also summarized the results obtained with colchicine treatment up to 1940. Here only those reports which have a bearing on the problem described will be mentioned.

In normal animal germ cells Nebel and Ruttle saw doubling of chromosomes in fertilized eggs of *Arbacia*. Pincus and Waddington found that rabbit eggs treated with colchicine became tetraploid. Lits (1933) used trypaflavine, arsenicals, mercury, zinc, iodine, bromine and certain toxins to study various tissues of the rat and found that these chemicals induced pycnosis and atypical cell division. An abundance of mitoses was noted in these tissues, 16 hours after injection of 0.025 mg. of colchicine into the rat. These cytological studies were followed by Allen in 1936 who applied colchicine in the study of cellular activity in the endocrines. Nebel (1937) and Nebel and Ruttle (1938) studied the nuclear changes in the stamen hairs of *Tradescantia*. Gavaudan and Gavaudan (1937) treated plants with colchicine and reported arrest of growth with 1/1,000–1/5,000 solution. They observed an increase in chromosome number in the cells studied. In a later paper (1938) they noted that the limit of increase in the number of chromosomes was determined only by the difficulties of nutrition. This they believed could

be surmounted by cultivating plantules or polyploid cells in tissue culture. Gavaudan, P., (1938) studied seedlings of *Vicia faba* which were immersed for two hours, three days in succession in a 1/1,000 solution of colchicine. The plants grew slowly and showed diverse monstrosities of stomata and leaf structures. Tetraploid and even octoploid cells with numerous nucleoli were observed. These changes were associated with interference with the normal karyokinetic process.

Roots of *Allium cepa* were studied by Gavaudan, Gavaudan and Pomriaskinsky-Kobozieff (1937) as affected by colchicine. They found that 0.1 gm. to 0.2 gm. of colchicine per 1,000 cc. in water induced bulbous hypertrophies behind the meristem of the root tips. They reported large numbers of abnormal mitotic figures 40 hours after treatment. Pseudoanaphase stages were noted in which the chromosomes were vaguely arranged in a quadrilateral form (pseudotelophases) with the two chromosomal groups united by a bridge of chromosomal material.

Eigsti (1938–1940), using the smear method, studied the effects of colchicine on the roots of onion, radish and corn seedlings. He found that the cytological changes were determined by the concentration of the colchicine solution, the time of exposure and the activity of the embryonic cells at the time of treatment. Eigsti (1940b) pointed out that chromosome breakage is higher in colchicine-treated material than in untreated cells of the same species. This, he inferred, induced variation other than polyploid changes.

Levan (1938–1939) made studies on the cytological effect of colchicine in the root mitoses of *Allium fistulosum* and *A. cepa*. Levan, too, observed that treated roots ceased to elongate but formed swellings at the root tip meristem, which he described as tumors. The volume of the meristematic cells increased but no new cells were formed. Levan showed that the cells in treated roots transferred to water reverted to normal mitosis. In colchicinized onion root tips he counted 128 and 256 chromosomes. Concentration as low as 0.0055% colchicine caused disturbance of the spindle. The threshold value of colchicine on root mitoses of *A. cepa*, Levan believed, was reached with a 0.005% to 0.01% solution after an exposure of four hours. Nebel (1937) found a 0.004% solution of colchicine effective in plants, while Ludford (1936) showed that a solution of 0.00001% was effective in ani-

mal tissue. Mangenot (1938a, b, 1939) studied roots of *Allium cepa* and *Hyacinthus orientalis* and seeds of *Lupinus albus* grown in Knopf solution to which 1/2,000 to 1/3,000 colchicine was added. He found that the roots remained alive after a long period in the mixture. He observed the tumefaciens effect of colchicine and ascribed it to a defect in the growth of the roots. The number of prophase stages was studied by Mangenot who contended that the number of cells in this stage did not vary up to 102 hours of exposure to colchicine, after which it decreased slowly, while the number of metaphases, anaphases and telophases fell off rapidly to zero. Levine and Gelber (1943) showed that with a colchicine concentration of 0.01% the maximum number of metaphase stages in the root tips of *Allium cepa* was reached after an exposure of 24 hours. Continued exposure to the same solution caused a general reduction in the number of metaphase stages until the number reached in the 140th hour was smaller than that found in normal root tips.

Shimamura (1939a) investigated the effects of colchicine on the roots and seedlings of *Allium cepa*, *Lycopersicum esculentum* and *L. pimpinellifolium*. He exposed the roots of onion to a 0.4% solution for two hours, after which they were placed in running water for one hour and then fixed at varying intervals. Shimamura described in detail the chromosome behavior in the colchicine-treated onion root tips, and believed that colchicine interfered with the mechanism that pulled the chromatids apart. In the absence of spindle fibers Shimamura described an orientation of the chromosome material about a degenerate spindle substance. The chromosomes became vesicular, they approached each other and formed a dumbbell-shaped body. The two masses of chromosomes connected by a narrow bridge of chromatic material separated at the link and formed two nuclei. In some cases the chromosome masses fused, forming a tetraploid nucleus. The tomato seedlings treated with colchicine produced tetraploid and myxoploid plants. In the roots of these seedlings aneuploidy was seen. Duhamet (1939) attempted to establish the colchicine concentration which would be most capable of producing a reaction in the roots of white lupine grown *in vitro* and attempted to induce a diminution or suppression of these effects. He observed that colchicine in concentration 10^{-4} completely inhibited growth of isolated roots in 10 to 12 days. Ad-

dition of heteroauxine in concentration 10^{-12} to this solution of colchicine interfered with the growth block induced by colchicine and was followed by a return to normal division. Levine and Lein (1941) studied the effect of various growth substances, *e.g.*, vitamine B, indoleacetic acid and colchicine, on root number and length of roots of *Allium cepa* grown in water in which these substances were used singly or in combination. Colchicine 10^{-3} solution inhibited root growth; when followed by exposure to a 10^{-8} solution of indoleacetic acid, stimulation of both formation and linear growth of roots occurred.

COLCHICINE ON THE ENDOCRINES

Allen used colchicine as an aid to determine the cellular activities of various endocrine tissues under normal conditions and under the influence of various hormones. He maintained that the colchicine technique was a new tool for the study of hyperplasias. Allen and Creadick (1937) studied the mitotic activity in the germinal epithelium of mouse ovary during sexual maturity by the use of colchicine. The arrested mitoses delayed the processes so that an adequate account of the activities could be determined. The effects of pituitary extract, prephysin and pregnant mares serum on the germinal epithelium were so interpreted. Allen and Creadick pointed out that the evidence was accentuated by the arrested mitoses after colchicine and that ovogenesis was clearly demonstrated to occur in the ovaries of the mature mouse at oestrus. Allen, Smith and Gardner (1937) studied the effects of theelin on genital tissue of the mouse after ovariectomy. Hyperplasia of the uterus muscle as shown through the retarded mitotic changes induced by colchicine was reported by Allen, Smith and Reynolds (1937). Mitotic figures in the vaginal epithelium of a colchicinized mouse numbered 1,000, while in the untreated animal only 10 to 20 dividing cells could be seen in a single cross section, as shown by Allen (1938). The efficacy of various methods of making vaginal smears could best be shown by the use of colchicine in the rat, as reported by Rogers and Allen (1937). Williams, Stein and Allen (1941) studied the genital tissues of the female mouse after application of colchicine. Colchicine effects were noted in six hours in the uterus and rectum. No effect was observed in spayed mice not treated with oestrone.

Following Allen and his associates, Bastenie and Zylberszac (1937a–d) used colchicine to arrest mitotic activity to better study the effects of the extracts of the hypophysis on the thyroid in young guinea pig, the influence of testosterone propionate on the genital apparatus of the rat and the effect of extracts of the antepituitary on the reproductive organs of the immature female guinea pig. The stimulative effects of antehypophysis on the parathyroids in the rat were demonstrated by the delaying action of colchicine by Bastenie and Zylberszac (1938). Leblond and Allen (1937) studied the effects of prolactin on the crop glands of pigeon by the use of colchicine. In a later paper, Leblond and Segal (1938) reported the effects of colchicine on adrenalectomized rats. The adrenal, he contended, suppressed the effects of colchicine. The lethal dose was one-third as strong for the adrenalectomized animal as for the control. Freud and Uyldert (1938) compared the intestines of normal and adrenalectomized rats after injection with cortin. The delayed nuclear division after colchicine injections showed that adrenalectomy had no effect on mitoses in the epithelium. Adrenalectomy did not interfere with mitotic activity in the intestine. Jailer (1938) applied colchicine in an effort to determine the mitotic index in the interstitial cells of guinea pig under the influence of the gonadotropic hormone. Bureau (1939) found, with the aid of colchicine, that oestrone and progesterone augment each other in their effect on the epithelium of the uterine horns of rabbit, as shown by mitotic increase. Androgenic treatment applied to castrated rats was studied by Burkhardt (1939) to determine its effects on the ventral prostate. Testosterone propionate was injected in 80-day-old castrated animals. The mitotic activity in the ventral prostate appeared at its best six hours after the injection of colchicine. Schmidt (1942) studied the ovary of the normal mature guinea pig after treatment with colchicine. Increase in mitoses was observed in the granulosa and theca interna and theca externa. The follicules were also increased in size. DuBilier and Warren (1941) studied the mitotic activity in the Brown-Pearce rabbit epithelioma with application of single and repeated small doses of colchicine. The number of mitotic figures for a given dose and given time after injection varied so that in general the epithelioma showed no consistency in its response to colchicine.

IRRADIATION WITH X-RAYS

It has been generally conceded that x-rays and the B and Y rays of radium are valuable adjuncts to surgery if not self-sufficient agents in the effective treatment of neoplastic diseases of man. Many roentgenologists believe that the efficacy of these rays lies in their destructive action on dividing cells. The resting stages have been considered resistant. However, there are two principal groups of investigators whose studies deal primarily with these highly important problems. Strangeways and his associates, Oakly and Hopwood, observed that irradiated tissue cultures showed a decrease in the number of dividing cells. They observed that this decrease was due temporarily to inhibition of the onset of mitoses. These views were supported by Kemp and Juel. On the other hand, Mottram, Richards, Grasnick, Holthusen, Alberti and Politzer and many others contended that cells are most sensitive to irradiation while in the process of mitosis. Love's (1931) studies on choroid and sclerotic tissues of embryo chicks of eight to nine days attempted to analyze the effects of x-rays on dividing cells. He studied the percentage of survival of dividing cells immediately after irradiation, and concluded that reduction in the number of cells in mitosis after irradiation was due to inhibition of some factor of those cells which normally would have entered mitosis during irradiation. He stated that the radio-sensitivity of a cell was a function of its displacement from maturity. When the displacement of a cell from its maturity was less than 100 minutes, the radio-sensitivity was constant and independent of displacement. When the displacement of a cell from its maturity was greater than three hours, its radio-sensitivity decreased. Studies on the effects of chromosomes of animals and plants have been numerous. Here, only the recent literature dealing with the application of irradiation to onion root tips will be stressed.

Nichols (1941), working with Sax, investigated the chromosomes of untreated root tips of onion seedlings. He reported that chromosome aberrations were frequent in very young seedlings, while in older ones few chromosome abnormalities were found. Nichols believed that cells with chromosome aberrations were eliminated by differential survival of the normal and abnormal cells. Marshak and Hudson (1937) used onion seedling root tips as a biological method of measuring roentgen ray dosage. They be-

lieved that the biological response per roentgen (r) is independent
of wave length over the region studied. They used small doses,
40r to 346r. Marquardt (1938) studied the effects of soft rays on
Scilla campanulata root tips and pollen mitosis in *Bellevalia romana*.
He described two kinds of effects which he called primary and
secondary. The first effect occurred in such nuclei as, at the time
of irradiation, were found in mitosis or about to enter that state.
The primary effects were described as clumping and fragmentation
of chromosomes, lack of spindle formation and indefiniteness of
division polarity. The period following the primary effects was a
mitosis-free interim after which the secondary effects became ap-
parent. In the pollen studies there was no mitosis-free interim.
The secondary effects brought about chromosome injuries, frag-
mentations and restitution processes, lateral chromatid transloca-
tions, ring-forming injuries and inversion forms. Villars (1940)
studied the effects of x-irradiation on colchicine-treated roots of
Pisum sativum and *Allium cepa* seedlings. They were placed in a
solution of 1 : 2,000 colchicine and irradiated after 40, 48, 96 and
120 hours of immersion. Their treatment had no effects on stages
up to and including prophase. In the following stage the chromo-
somes agglutinated and fused into one mass, and spindle formation
was inhibited.

Sax (1941) studied the effects of x-irradiation on the roots of
Allium cepa. While the primary purpose was to describe the chro-
mosome changes induced by the x-rays, an exposure to 0.1% col-
chicine for 24 hours was applied to facilitate this study. Aberration
frequency was based on the number of chromosomal abnormalities
per hundred cells counted. Sax described primary and secondary
effects produced by x-rays which were in accord with Marquardt's
observations. The clumping and fragmentation of chromosomes
after the treatments given by Marquardt support the contention of
Dustin and his workers that colchicine had a radiomimetic effect.
Sax's experiments did not differentiate between the effects induced
under colchicine and those by the x-rays. Sax showed that high
x-ray doses suppressed nuclear division from one to two days, while
low x-ray doses retarded nuclear activity and the chromosome
clumping persisted for several days. The primary effects were evi-
dent, Sax claimed, on the metaphase stage. The secondary effect
of x-rays involved the production of typical chromosomal aberra-

tions which consisted of chromosome breaks, usually followed by chromosome union to produce rings, dicentrics, inversion, transloca- tions and interstitial deletions. The secondary x-ray effects, Sax believed, persisted for several cell generations, but were soon elimi- nated, and cells with normal or balanced genoms survived. Sax used doses of x-rays from 150r to 600r. Larger doses of 1,500r to 5,000r were tried on seedlings of ornamental plants; the highest dosage killed or retarded the seedlings of most species studied.

SUMMARY

It has been shown that colchicine administered or applied in small doses to plants or animals produces a retarding effect on the mitotic process of cells in either growing vegetative or germinal tissue. The effect is not one of growth stimulation but one of growth delay with resulting hypertrophy of the affected cells. The nuclei in these cells fail to complete the division process, for they become arrested after the chromosomes divide (metaphase stage). In colchicine-treated mammalian tissue the metaphases begin to ac- cumulate in two to three hours and reach the maximum number in ten to fifteen hours. In cold blood animals, the metaphase stages gather much more slowly and the maximum number occurs after five to seven days. In roots of *Allium cepa* the metaphases reach a maximum after an exposure to 0.01% solution in about 24 hours.

Hypertrophy of the cells apparently begins soon after the meta- phase stage is reached. After the 24-hour exposure the cells with their doubled number of chromosomes begin reconstruction of their nuclei and atypical ones are formed. This phenomenon becomes very prominent after 48-hours exposure to this drug. At this con- centration of the alkaloid, death is not produced, for the roots recover when they are washed thoroughly and immersed in fresh tap water. The hypertrophies, however, do not disappear and growth of the meristematic tissue is resumed.

The area of a plant inoculated with the *B. tumefaciens,* when cov- ered with a lanolin paste to which colchicine is added, does not prevent this plant tumor from developing. When this paste is ap- plied to a well formed gall, death of the overgrowth results.

Malignant tumors of animals are characterized by numerous cells in nuclear division. Injection of colchicine, in appropriate solu- tion, into the cancerous animal emphasizes the division phase in the

cells of the tumor. The many chromosome aberrations found in malignant cells (Levine, 1931) are analogous to those induced by colchicine. The young diploid peripheral cells become arrested in metaphase, and hyperchromatic cells typical of the older parts of the cancer are produced.

The now well established facts that colchicine arrests nuclear division in a stage where the chromosomes are prominent features of the cells, and that these chromosomes remain in the cells as distinct bodies for longer periods of time and that many more cells attain this stage than can be found in untreated tissue, make the use of colchicine of great importance in the treatment of neoplastic diseases. It is accepted by some roentgenologists that the cell in division is more vulnerable to x-rays or radium than when in resting stage. Continued application of the poison in the treatment of this disease raises the colchicine level to the lethal point and causes death. Repeated application of x-rays and radium to deep seated growths has not been conclusively successful. The suggested rationale involves the use of both agents. Continued use of colchicine and x-rays has been successful in some animal work. In cancer of man insufficient tests have been made. The cases used were in terminal stages.

The report on the effect of combined colchicine and x-ray treatments to fundamental tissue like the root tip of onion, will appear elsewhere. The study on simple tissues should serve as guide for a more intensive study on animal tumors. Crown galls on economic plants may be treated more effectively by preventive methods which will avoid the introduction of the tumor-producing organism.

In endocrinology, colchicine has proved to be an aid in the study of normal growth processes, while in plant breeding the influence of colchicine on seeds and buds has been productive of some useful economic plants. Genetically, according to Blakeslee, colchicine has given the geneticist a tool for the production, at will, of new species of plants.

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THE BOTANICAL REVIEW

PLANT GROWTH RELATIONS ON SALINE AND ALKALI SOILS

O. C. MAGISTAD

U. S. Regional Salinity Laboratory, Riverside, Cal.

FOREWORD

Serious study of saline and alkali soils and their relation to plant growth was begun by Hilgard (125) before 1886 and later summarized in 1900 (126) and 1906 (127). It received a mighty impetus with the work of Gedroix (78, 79, 80, 81, 82, 83), de Sigmond (297, 298, 299, 300, 301, 302, 303, 304, 305, 306), Kelley (164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179) and Hissink (128, 129, 130, 131, 132, 133, 134, 135) who all demonstrated that alkali soils differ from normal soils in containing abnormal quantities of replaceable sodium. Harris (99), in his book in 1920, reviewed the subject of alkali soils, but this was prior to a general knowledge of base exchange in soils and many recent advances in plant physiology. Saline and alkali soils usually occur in arid regions where rainfall and leaching are insufficient to carry away the soluble salts.

On saline soils evidence indicates that plants are reduced in growth mainly because of the increased osmotic pressure of the substrate, while on alkali soils, usually with high pH values and oftentimes in the presence of sodium carbonate, plant growth is also reduced because of poor soil permeability to water, lack of oxygen in the soil air, malnutrition, chlorosis, and even corrosive action. These subjects will be reviewed later, but they indicate the need for a brief review of the various types of salty soils and their nature.

CLASSIFICATION AND COMPOSITION OF SALINE AND ALKALI SOILS

Formation

Clear-cut terminology and classification of salty soils has not yet been obtained. The processes which lead to formation of salty soils can be summarized as follows (11, 37, 80, 82, 166, 167, 187, 301) :

a. Salts usually accumulate in soils because neither the surface nor the ground waters drain away. With evaporation directly or by transpiration the salt concentration is increased. Sodium salts may predominate at the outset; however, with further concentration, calcium, because of the limited solubility of calcium carbonate and calcium sulfate, is usually precipitated out of solution with a further increase in the relative proportion of sodium. This process of salt accumulation is called "salinization" (167, 301).

b. An equilibrium exists between the distribution of bases in the soil solution and on the soil colloids. With higher actual and relative quantities of sodium present in the soil solution greater quantities of sodium are absorbed by the soil colloids with a corresponding release of other cations previously absorbed. This process, in which the percentage of sodium on the exchange complex is increased, is called "alkalization" (301).

c. With subsequent leaching and removal of soluble salts the soil colloids disperse because the coagulating effect of the electrolyte is removed. The process of salt removal is called "desalinization" (167, 301).

d. With desalinization (301) new equilibria between sodium in the soil complex and in the soil solution are set up, with loss of sodium from the complex. If the soil is calcareous or gypsiferous so that relatively large quantities of calcium are always present in the soil solution, calcium will exchange for the sodium removed from the soil complex, forming again, from a chemical standpoint, a normal soil. More often there is little calcium present in the soil solution, and sodium is hydrolyzed from the complex with the formation of small amounts of hydrogen clay and sodium hydroxide. The sodium hydroxide reacts with carbon dioxide from the soil air to produce sodium carbonate. The pH value of the soil usually rises to above 8.5. This process of sodium removal with hydrogen exchange is called "degradation" (301) and "solotization" (167, 301). With prolonged leaching the sodium carbonate is removed and hydrogen exchange may go so far that the pH values of the surface soil may fall below 6.0.

e. If a supply of soluble calcium is made available to a degraded soil, this calcium will replace the exchangeable hydrogen and again form a soil normal with respect to its exchangeable bases. The calcium is usually supplied by addition of agricultural lime (calcium carbonate). This process is called "regrading" a soil.

Classification

On the basis of the chemical changes involved de Sigmond (301, 304) classifies salty soils in the various stages of formation into:

1. "Saline soils" which contain over 0.1% salt and less than 12% of the exchangeable bases K + Na. This corresponds to phase (*a*) above; to Gedroix's term "Solonchak" (82) and roughly to Hilgard's term "white alkali". pH values of the soil at normal field moisture contents usually are below 8.5.

2. "Saline alkali soils" which contain over 0.1% salt and over 12% replaceable K + Na. This corresponds to phase (*b*) above. The value of 12% K + Na is taken provisionally because at about this range soil dispersion is serious, and the soil has a markedly lower permeability if leached. Soil pH values may slightly exceed 8.5.

3. "Slightly saline alkali soils" contain over 12% replaceable K + Na, and slightly over 0.1% salt. This type corresponds to (*c*) above and is produced by desalinization. These soils also often contain sodium carbonate. pH values usually range between 8.5 and 10.0.

4. "Degraded alkali soils" contain less than 0.1% salt and appreciable quantities of replaceable hydrogen. Soil pH values in the surface horizons may be below 6.0, but are usually between 6.5 and 8.0.

5. De Sigmond has also included a fifth class called "regraded alkali soil" containing a normal quantity of replaceable Ca + Mg and produced by process *e* above. Since this type of soil is again normal, it is no longer an alkali soil and should not be included as an alkali soil.

Kowda (187) has divided alkali soils into five classes based on salt content, carbonate content, presence of gypsum and location of the lime layer, while Antipov-Karataev (11) also includes organic matter.

To illustrate the difference in exchangeable bases between normal, saline and alkali soils, the following table has been prepared from data as indicated:

		Exchangeable bases Relative per cent				References
		Ca	Mg	K	Na	
Normal soil						
California soils	av. 7	63	25	4	8	169
Russian soils	av. 2	82	11	7	0	169
Holland soils	av. 26	79	13	2	6	130, 169

Saline soils					
California soils av. 9	67	26	3	4	170 (table 4)
Alkali soils (Western U. S.)					
Intermediate av. 5	43	21	10	26	170 (table 3)
Extreme av. 14	4	8	16	72	170 (table 2)

It will be noted that normal and saline California soils have approximately the same distribution of exchangeable bases. The saline soils differ from the normal in containing 0.2% soluble salts. Many authors take 0.1% as the threshold of saline soils, particularly if chlorides predominate.

Most other writers (82, 90, 127, 166, 180, 323, 340) have failed to break the soils down into as many classes as has de Sigmond. The Division of Soil Survey (47) recognizes saline soils and alkali soils approximately as defined above. Kelley (167) has objected to classifications such as that of de Sigmond's on the ground that one class merges into another. He has used Hilgard's nomenclature in which alkali is used in a generic sense to include all classes (1 to 4 inclusive) above. Hilgard (127) differentiated between "white alkali" and "black alkali". His white alkali would probably include classes 1 and 2 above, and black alkali classes 3 and 4. Gedroix (82) used the terms "Solonchak" to designate a saline soil without structure and "Solonetz" "a type of soil in which base exchange silicates contain appreciable quantities of replaceable alkali bases, chiefly sodium" (82). The degraded alkali soil is called "Solodi" by Gedroix on the basis of chemical characteristics. Antipov-Karataev (11), in a review of Russian work, classified solonetz soils as follows:

a. "Tschernozemartige Solontzi"—containing over 30% replaceable Na in the exchange complex of the illuvial horizon.

b. "Humus poor Solontzi"—containing over 20% replaceable Na.

c. "Solonetz-like soils"—not true Solonetz soils but which have many of their properties:

1. "Tschernozemartige"—3–20% replaceable Na.
2. "Chestnut colored"—5–20% replaceable Na.

Other Russian investigators were more concerned with soil classification and mapping from visual criteria (89, 340). Vilensky used the term "Solonchak" to denote a saline soil without defined structure, and "Solonetz" a salt soil with structure (178, 341). In the English language the meaning of the terms "saline" and "alkali" is gradually being restricted, and the author here uses "salty" in a

generic sense to include all soils (1 to 4 inclusive above); this is not a new use (301, 339). The Russians also use the root "solon" in this sense. De Sigmond has used the generic terms "salty" (301), "sodium" (304) and the Hungarian term "Szik" to include both saline and alkali soils. The terms "Reh" and "Usar" (India) (69, 125, 307) and "Brak" (South Africa) (329, 358) are also used in this manner, although (323) states that "reh" is a saline soil, "bara" an alkali soil and "bari" a partially alkalized soil. The Indian writers (323) indicate that alkali soils have a pH over 8.5 and the effect of high replaceable sodium exceeds the effect of soluble salts. Trietz (328) has found vegetation a good index to the nature of alkali soils.

In this review saline soils will be defined as class 1 above, while alkali soils will include those containing over 12% replaceable alkali bases.

Base Exchange Relations

It is now well established (9, 95, 118, 173, 174, 175) that the base exchange materials in a mineral soil are crystalline in nature. The best recent reviews of this subject are by Grim (95), Hendricks (118), Kelley (168) and Scheffer and Schachtschabel (286). These soil minerals are of three main types: montmorillonite, hydrous mica (illite) and Kaolinite. The first has a marked expanding lattice and a base exchange capacity of 60–100 milligram equivalents per 100 grams. The illite or hydrous mica group has a structure similar to montmorillonite but does not expand. It has a capacity of 20–40 m.e./100 grams. Kaolinite does not expand with varying water content and has a capacity of 3–15 m.e./100 grams (95). The spaces between the lattices of these crystals are large enough to permit easy entrance of the common soil bases in base exchange for exchange purposes (95). The size of these soil crystals is usually less than 5 µ in diameter. While it is well established that organic matter in soils has a high base exchange capacity (3, 83, 132, 222, 223, 224, 239, 241, 249, 251, 286, 349), little is known of the mechanism responsible. Base exchange capacities of organic matter as great as 431 m.e./100 grams are reported (223).

There are two principal methods of determining the nature of the base exchange crystals in a soil: by means of X-ray diffraction (121, 173, 177) and by differential thermal analysis (120, 177). Kelley, Dore, and Page (174) have reported that saline (white alkali) soils

of the West contain all three types of soil minerals, while the alkali
soils (black alkali) contained chiefly mica-like minerals. The
methods used by them have been criticized, as they did not initially
saturate the soils with calcium or control the hydration. Aldrich,
et al. (8) indicate that this is necessary because with sodium as the
adsorbed base and lack of moisture control the diffraction lines of
montmorillonite are almost identical with those of hydrous mica and
the mineral may easily be mistaken for hydrous mica.

The soil minerals present in alkali soils are believed to be largely
montmorillonite and hydrous mica (9, 119, 292).

Composition of Salts in Saline Soils

De Sigmond (304) classifies the salts in saline soils on the basis
of the nature of the anions present into: soils with (a) sulfates, (b)
chlorides, (c) sulfates plus chlorides, (d) sulfates plus carbonates,
(e) chlorides plus carbonates, (f) chlorides plus sulfates plus car-
bonates, and (g) soda soils. The farmers of India also have spe-
cific names for the various salt types (69). It appears doubtful that
sodium carbonate can be present in any quantity in soil solutions,
and the soil remain a saline soil (below 12% K + Na). It would
appear that if soluble carbonates are present in the soil it is a saline
alkali or alkali soil. Classification of saline soils on the basis of the
cations present rather than the anions would have many advantages,
especially in base exchange equilibria studies.

Kelley and Brown (170) list analyses of nine saline soils (those
containing less than 10% replaceable sodium). The total soluble
salt content ranges from about 2,000 p.p.m. to about 60,000 p.p.m.
Sodium is the predominant cation in most cases, and chlorides or
sulfates are the predominant anions. No soluble carbonates are
present which could be titrated with phenolphthalein as an indicator.
These analyses are typical for saline soils (37, 48, 127, 203, 213,
291, 313).

In some localities sodium may not be the predominating cation;
thus analyses are reported where calcium exceeds sodium and where
magnesium exceeds sodium (48, p. 73).

Chlorides and sulfates are the predominant anions in saline soils.
In certain regions, e.g., the Pecos River Valley of New Mexico and
Texas (291), the Billings area of Montana (48, p. 109), the San
Luis Valley of Colorado (48, p. 117), Alberta, Canada (256), and

parts of Hungary (302), sulfates markedly predominate. Occasionally nitrates may be present in large quantities (10, 190). One analysis is listed where 5,000 p.p.m. of nitrate were present in the soil and far exceeded the quantities of other anions (170). Humid soils may sometimes contain excessive nitrates (42).

Chemical Nature of Alkali Soils

In normal soils calcium plus magnesium constitutes 90% to 95% or more of the replaceable bases (11, 139, 169). The predominant characteristic of an alkali soil is that the alkali bases, sodium and potassium, constitute more than 12% of the total replaceable bases, when calculated on an equivalent basis. De Sigmond (301, 305) is probably the only author who has specifically mentioned 12%, but most authors on the subject would put the value not much above 20% (11). De Sigmond arrives at this value because when soils contain more alkali replaceable bases, they become dispersed when leached (94). An inspection of the data from (266) indicates that pH values rise above 8.5 when the replaceable sodium percentage exceeds 14. Fireman and Magistad (62) have unpublished work which also indicates a decided decrease in permeability of soils when replaceable sodium exceeds 10–15% of the sum of the replaceable bases. The sum of replaceable bases in milliequivalents per hundred grams is commonly designated by the letter S (132).

While some (265, 301) include replaceable K with replaceable Na, the amount of replaceable potassium found in soils is usually only 1% to 5% and is ignored. Occasionally the value may be great. Thus Kelley (170) lists analyses where K constitutes as much as 40%, 11% (324), and 30% (189) of total capacity.

Magnesium-saturated soils are said to have some of the physical properties of sodium-saturated soils (248, 312). Replaceable magnesium constitutes a large portion of the base exchange capacity in some soils of solonetz structure (60, 167, 180, 189, 248, 280, 281, 320), but whether it is the cause of such structure or only remains after sodium salts have been removed, is unknown.

Definite equilibria exist between the bases in solution and the replaceable bases on the soil minerals (14), which has been represented by absorption isotherms (151, 357) but more accurately by mass action equations (182, 259, 335), particularly that of Gapon (12, 70, 211).

Analysis of Soils for Soluble Salts and Replaceable Bases

Soluble salts can be readily determined with fair accuracy in soils which do not contain calcium carbonate, gypsum or much replaceable sodium (95). Where gypsum is present the amount of calcium and sulfate ions found will depend upon the amount of water used to remove the salts (95). In the presence of considerable replaceable sodium the equilibrium between calcium and sodium in the solution and on the colloid varies with the water content; hence here, too, unless one obtains the soil solution at normal moisture contents, errors are introduced (29, 78, 83, 95). In the presence of replaceable sodium and calcium carbonate, addition of water shifts the equilibrium so that replaceable Na is released and sodium carbonate formed (29, 57, 78). For this reason many of the older analyses in the literature on the quantities of sodium carbonate in soils are very questionable. Sodium carbonate does exist in many alkali soils, but in alkali soils containing calcium carbonate or gypsum the amounts reported are usually too great (243). In the same manner difficulties arise in the determination of replaceable bases. One cannot leach out the soluble salts and expect to retain the replaceable bases unaltered (72, 129). In the presence of calcium carbonate and gypsum the replacing reaction does not go entirely to completion, and, furthermore, when the soil is leached with a reagent to remove the replaceable cations, this solution contains in addition some dissolved calcium from the calcium carbonate, gypsum and soil minerals. More satisfactory methods for determination of replaceable bases in the presence of gypsum are needed.

The replaceable sodium content of alkali soils may nearly reach 100% of the total capacity (170, 265, 268, 362).

PLANT RELATIONS TO SALINE SOILS

Plant relations to saline and to alkali soils will be discussed separately. The present section will be limited to saline soils containing the common salts, *i.e.*, combinations of the ions Ca, Mg, Na, Cl, SO_4, HCO_3 and NO_3. (Alkali soils contain more replaceable sodium than saline soils, often contain sodium carbonate, and usually have pH values above 8.5.)

Western soils are usually fertile mainly because they have undergone little leaching. Most western soils require additional nitrogen, and some, especially in the Northwest, boron and sulphur. As a

rule, therefore, there is little deficiency of phosphorus, potassium, calcium or magnesium, although phosphorus is becoming increasingly deficient. On the other hand, in saline soils there is usually an excess of calcium, magnesium and occasionally of potash, nitrates and boron (207). It seems best to review the literature on plant relations in saline soils on a causative basis rather than chronologically. Many of the pieces of work can now be interpreted in the light of present knowledge and new over-all conclusions reached.

The older investigators searched for toxic substances or toxic concentrations, but more recent work indicates that with increasing salt content growth is usually merely inhibited.

Growth Reduction Rather Than Toxicity

Eaton (54, 56) was one of the first American writers to point out that when grown in a saline solution, plants are merely reduced in growth without any marked change in outward appearances. He stressed the fact that there is no toxic limit short of which healthy plants are produced and which if exceeded, causes death. This view has been substantiated by more recent work (15, 46, 49, 74, 76, 112, 114, 209, 210, 247), and re-examination of older work points to the same conclusion (161, 216, 221, 331, 332). A large number of investigators have reported reduced growth at concentrations somewhat in excess of two atmospheres osmotic pressure when compared to lower concentrations (15, 17, 46, 49, 56, 65, 73, 74, 75, 77, 91, 110, 112, 114, 116, 136, 195, 209, 216, 221, 240, 247, 252, 294, 311, 318, 319, 327, 331, 338, 345, 347, 356), and this growth depression is roughly proportional to the increase in osmotic pressure. In some instances approximate osmotic values have been calculated by the author for the above experiments. A number of writers believe that it is the individual action of certain ions in salt solutions which is the cause of reduced growth rather than the osmotic effect. This is particularly true of certain very toxic or corrosive ions like CO_3^{--}. The fact that approximately equal growth reductions are obtained at the same osmotic level, when produced by the common salts of saline regions, lead most people to accept the osmotic theory. This will be discussed further throughout the paper.

The optimum concentration for growth varies with the crop and experimental conditions. Where aeration is provided for, and there is a plentiful supply of nutrients obtained by frequent changes of

solution, maximum growth is often obtained at concentrations below
one atmosphere (31, 75, 114, 136, 236, 247, 347, 348).

Osmotic Effects and Water Relations

The plant absorbs water because the osmotic pressure of the root
cells is greater than that of the soil solution, according to Maximov
(219) and others (321). The turgor pressure of the cell wall modi-
fies the osmotic pressure of the cell contents so that the net effect
governing water intake is best described by a term such as "diffusion
pressure deficit (237). In this paper osmotic pressure of plant
parts has this overall meaning. If the concentration of the soil solu-
tion is increased, the osmotic pressure gradient is decreased and
water absorption goes on at a slower rate. The plant, however, can
in part accommodate itself to such a condition by increasing the
osmotic pressure of the cell sap (52, 54, 55, 56, 112, 146, 219). Salt
marsh plants and desert plants in general have saps of high osmotic
pressure, and J. Arthur Harris and co-workers carried on much
work to correlate the plant sap concentration with that of the sub-
strate (50, 63, 100, 101, 102, 103, 105, 106, 107, 219 p. 276, 351).
In a number of instances cell sap concentrations, as determined by
the freezing point method, were found to exceed 40 atmospheres
(101, 146), although sap of agricultural plants usually had an
osmotic pressure ranging from 10 to 20 atmospheres (56, 146).

The osmotic pressures of soil solutions from saline soils vary with
the amount of salts present, the water content, the nature of the salts
and the nature of the soil. In any particular soil the soil solution is
most concentrated, as far as the plant is concerned, when the mois-
ture content is reduced to the wilting percentage (28). Magistad
and Reitemeier (213) reported the osmotic pressures of such soil
solutions to be in the neighborhood of one to two atmospheres for
normal productive soils and as high as 200 atmospheres or more for
barren saline soils. Poor crop growth was obtained with soils hav-
ing osmotic pressure of 10 atmospheres and none at 47 atmospheres.
The soil moisture content may never be depleted to the wilting per-
centage and at maximum soil moisture content (i.e., field capacity)
the osmotic pressure of the soil solution is only about half that at
the wilting range. Vikulina (338) reported similar results.

High osmotic pressures of the soil solution are not restricted to
western saline soils. Heavy applications of soluble fertilizers often

increase the salt concentration of the soil solution to a degree injurious to plant growth, especially on sandy soils. Thus fertilizer applications of 1,200 pounds per acre on Norfolk sandy loam increased the osmotic pressure of the displaced soil solution to about 14 atmospheres (359). Fertilizer applications at rates as great as this are occasionally made.

To investigate the effect of osmotic pressure of substrate on water absorption by plants, Eaton (55) placed a part of a root system in a dilute solution and part in concentrated solution and showed that water absorption was mainly from the dilute solution. Similar experiments with approach grafted tomatoes (199) resulted in decreased water intake from the concentrated solutions as well as from solutions having high osmotic pressures as a result of additions of salts or sugars. The most direct measurements are those which attached potometers to *Allium sepa* and corn roots, respectively, and measured water intake (113, 279). For non-conditioned intact plants Hayward and Spurr obtained the following rates of water intake at 10 cm. from the root tip:

			Water intake $mm.^3/mm.^2/hr.$
control	−	.8 atm.	0.249
control	+	2 atm.	0.075
control	+	4 atm.	0.030

The rate of water absorption is of course also related to climate conditions and the rate of transpiration. As Maximov (219) has pointed out, the shoots of desert plants and halophytes are adapted to decrease the rate of transpiration yet maintain sufficient area to take in the necessary carbon dioxide and provide for photosynthesis.

One group of men (16, 198, 311a) in their work and reviews indicate that normal vegetative growth and succulence is associated with tissues which have a high colloid and moisture content and a plentiful supply of nitrogen. If the moisture content drops, the soluble salts within the cell may exceed critical values and be partially or wholly precipitated or salted out. This is apt to happen during periods when water demand is high, as in flowering or during periods of hot dry weather. Older leaves can not recover once their colloids have been partially dehydrated, and thereafter they will have a lower water content and may die. Thus Bakhuyzen (16) believes that annuals die after flowering because they have lost so much water at the time of flowering that the plant can not recover.

A somewhat different view is presented by Iljin (146) and Maximov (219, 220). They postulate that with decrease in water content the cell contents and protoplasm shrink away from the cell wall. Once there is a separation at this point, water can not be conducted to the protoplasm and permanent wilting ensues. Some plants like sunflower, according to Maximov (219, p. 313), can lose as much as 25% of water and recover, while some shade plants can not recover if they lose 2% to 3% of water. This difference in behavior he ascribes to the elasticity of cell walls in the case of plants like sunflowers which permit the cell wall to follow the contracting protoplasm.

Caldwell (38), working with four plant species, noted large decreases in per cent moisture of leaves of plants when grown in nutrient solutions of several atmospheres. Similar data were obtained for barley (73) and flax in calcium chloride solutions, but not in substrates with sodium chloride and sodium sulfate (114). According to the theories given above, the plant in saline solutions will first have a water loss which is not recovered in the older leaves. Hence these leaves thereafter will have a lower moisture content. Newer leaves and parts may have a relatively high water content.

Soluble organic materials, especially sugars, also contribute to the osmotic pressure of plant cells. Decrease in water content in some way increases enzyme activity and hydrolysis of carbohydrates to simple sugars.

Henchel and Kolotova (117) classify halophytes into three groups based on the mechanism for salt tolerance, i.e., those with great tolerance or endurance to the increased salt content within the cells, those that can excrete or eliminate excess salt, and those that have a low permeability of protoplasm to salts. Under the first category would be included those plants which can tolerate high osmotic pressures in the cell sap; some examples are given in which these pressures exceed 40 atmospheres (101, 219, p. 19). The other two categories will be discussed under the section Soils of Low Permeability.

The view that salt tolerance is largely a matter of reduced water uptake has been retarded by the fact that many have interpreted these same experimental data on the basis of antagonism, and by the wide-spread belief that plants do not suffer from lack of water at soil moisture contents above the wilting coefficient. Several investigators have plotted daily growth or transpiration against time

and have noted no perceptible decrease in this rate before the permanent wilting percentage was reached (122, 123, 336, 337). From this they concluded that water is equally available to the plant from field capacity to the neighborhood of the permanent wilting percentage. Since these experiments were usually conducted in soils of low salt content, investigators ascribed reduced growth rates in the presence of salt to the salt *per se*, and considered that water availability was not involved to any appreciable extent.

The work of Briggs and Shantz (32, 33) and of more recent authors has indicated that all plants wilt at about the same moisture content in any soil, or in terms of tension units, at about 15 to 20 atmospheres. Since less than 10 atmospheres of added salt will in many instances cause death of plants in sand cultures, this was considered additional evidence that the plants did not die for lack of water but because of some action of the salt. With advances in the field of soil moisture relationship the stage has recently been set for clarification of these views.

Day (45) and later others (21, 58) postulated from thermodynamic reasons that the moisture potential of a soil is equal to the sum of the pressure potential plus the osmotic potential. That is, the free energy of the water in the soil, and hence its relative availability to plants, is proportional to the forces holding the water to the soil plus the osmotic forces of the dissolved salts, and to be additive, they must both be expressed in similar units, such as ergs per gram of water. Physicists have used the unit ergs per gram of water when measuring potentials. Other soil moisture and plant investigators have not been as precise and have discussed the forces on an area basis rather than a mass basis. Thus much of the data in the literature has been expressed in atmospheres, or tensions per unit area, and these terms are more familiar to plant men. Under standard conditions one atmosphere = a tension corresponding to a pressure head of 1,029 cm. of water and corresponds to a potential of 1.01×10^6 ergs per gram. On this basis the wilting range of soils has been found to be about 15 atmospheres (277, 321) and 10 to 23 atmospheres (58). Thus plants will be unable to obtain water rapidly enough to maintain life when the moisture stress reaches about 15 atmospheres, whether because of a drying soil, or the presence of salts, or both. That this conception has considerable support is evidenced by the data (277) of the best correlation between

moisture potential and wilting range when osmotic potentials were
included, and by others (15, 58, 213). Webster and Viswanath
(356), working in Iraq, state that plants wilt more quickly in a
salty soil than in the same soil with the salt removed. They also
suggest that a soil is too saline for growth of wheat and sorghum
when the osmotic pressure of the soil solution to a depth of six feet,
determined at the minimum capillary capacity, exceeds six atmos-
pheres. According to this concept, plants have greater difficulty in
obtaining water at moisture contents nearing the permanent wilting
percentage than at field capacity (188, 345), but the differences are
not readily demonstrable. Wadleigh and Ayers (345) have recently
presented additional evidence and have reviewed this subject.

Many workers have noted the relationship between drought-resis-
tant plants and salt-tolerant plants, and these two branches of study
were at one time united under Alkali and Drought Investigation in
the Bureau of Plant Industry. There are of course many distinc-
tions between the two; thus some plants are drought-resistant be-
cause of their deep root systems but may be quite sensitive to salt.
Nevertheless, a consideration of water requirements and the availa-
bility of water in terms of free energy or some similar concept will
do much to clarify the similarities and differences between salt
tolerance and drought tolerance.

If the salt had no action beyond decreasing water availability we
would expect plants to wilt at a certain water tension in a salt-free
soil of about 15,000 cm. water per sq. cm. or approximately 15
atmospheres. Here the osmotic potential would be close to zero.
In a sand culture solution the pressure potential would be nearly
zero (the water is not held by the sand with any appreciable force)
and the moisture stress should be approximately equal to the osmotic
potential. We would then expect plants to die in a salt solution of
15 atmospheres osmotic pressure. It is too early to tell if this rela-
tionship holds. Almost certainly there are some salt effects in addi-
tion to water retention effects. If the relationship did hold, we
would expect to find some plants having wilting coefficients of 20
atmospheres or more, and others probably below 10 atmospheres.
This is not in accord with the customary interpretation of wilting
coefficients. Yet an examination of the data of Briggs and Shantz
on relative wilting coefficients (32) shows *Colocasia*, hydrophytes
and xerophytes had values substantially greater than the others;

the difference undoubtedly exceeded probable error. Thus even the data of Briggs and Shantz would indicate that possibly all plants do not wilt at the same moisture tension. Part of this may be caused by lack of a well branched root system. Now that we know the nature of the force-moisture content curve (278, 285, 345) it is clear that differences in moisture content between 10 and 20 atmospheres are difficult to determine by soil sampling because of the steepness of the curve in this region and the magnitude of the sampling error.

It should be borne in mind that the plant is not static and that plants from the same original group will have higher internal sap concentrations when grown in salt solutions than when grown in more dilute solutions (56, 146, 311). Thus these plants tend to become adapted to their surroundings in such a way as to maintain an osmotic gradient favorable to water intake. The same is true of plants conditioned to cold.

Several authors have worked with bacteria rather than larger plants and have noted the relationship between osmotic pressure of substrate and growth depression (17, 93).

The electrical conductivity of soil solutions or substrates also correlates well with osmotic pressures of such substrates (43, 51, 209, 236), and in field practice it may be more convenient to determine electrical conductance rather than osmotic pressures as an index of the amount of salt present and of the unavailability of water to plants.

Salt Absorption and Accumulation

The older literature on permeability, antagonism and salt absorption is far from clarifying. Fortunately this subject was recently discussed and reviewed in 1940 by a group of biologists in a symposium at Cold Spring Harbor (262), and with special reference to agricultural plants (138), by Hoagland in his book (139) and in The Botanical Review (137). Many of the studies on salt accumulation have been made on large-celled marine forms, on potato discs, and some on excised plant roots, and on many points the results of researches on such varied materials are in agreement. Only the main points will be reviewed here with particular emphasis on plant relations in saline soils.

The concentration of ions, as a whole or singly, is usually greater in the cell sap than in the soil solution. Absorption is therefore against a gradient which may be very great. Thus Hoagland and

Davis (142), working with *Nitella,* showed that the concentration of K in the sap reached over 50 milliequivalents per liter when grown in pond water of 0.051 m.e. K per liter or absorption took place against a concentration gradient of 100. Similar gradients for other ions were Cl—100×, SO_4—30×, Ca—15×, Mg—6×, and Na—65×. The total concentration of sap in these *Nitella* cells reached the very high value of 173.5 m.e. per liter. Similar results have been obtained with *Chara* (40). Excised barley roots can accumulate K to a level of 20 to 50 m.e. per liter in the cell sap under proper conditions (141). Similar high cell sap concentrations have been found in tomatoes (112) and for various other crops (56).

Jacobs (150) has pointed out that accumulation is proportional not to the concentration of that ion in the external medium but to the differences in concentration inside and outside the cell. Thus a cell which is depleted or in a low state of supply as regards a particular ion will absorb that ion more readily. From the standpoint of gradients a cell can more easily absorb the essential ions from saline solutions than non-saline because the gradient is less.

Oxygen Supply

Work is performed in transporting ions against a concentration gradient, and the energy for this comes from oxidation of carbohydrates. The amount of oxygen required varies with the type of tissue (40, 258). While saline soils are usually "open" compared to alkali soils, they may often have low contents of oxygen in the soil air and thus restrict root activity. Of particular interest are recent papers (22, 23, 24, 25) which suggest that oxygen content of soil air is related to root activity of apple trees about as follows:

Per cent oxygen	
0.1– 3	roots merely subsist
5–10	root tips grow
10–12	water absorption proceeds
over 12	new roots form

Recent other work (342) has substantiated the correlation between poor cacao yields and low oxygen contents in the soil air. Magistad (in unreported work) observed yellowing of alfalfa in August when soil moisture tensions remained less than 100 cm. water for only 10 days, but during December and January, when temperatures were low and growth was slow, the alfalfa was not adversely affected by soil moisture tensions less than 100 cm. of

water for six weeks. Oxygen contents were not known, but at moisture tensions below 100 the soil is nearly saturated with water. Furr and Aldrich (66), working in a saline alkali soil, found that oxygen contents between 0.4% and 2% for three weeks did not result in measurable injury to date palms. Rice has been preferred as a crop during reclamation of saline lands and will grow with roots submerged (204). It has a relatively low oxygen requirement and is able to transmit air through the stem to the roots, as in several other plants (88, 158).

Water absorption is also facilitated by oxygen supply (24, 244, 360). Newton found that barley respired more and used more energy to obtain water from solutions of high osmotic pressures (244). His series of nutrient solutions ranged from 0.1 to 6 atmospheres. It is believed that strawberry clover does well on saline soils primarily because of its ability to tolerate water-logged conditions (74).

The preceding sections have discussed ion intake as though it were a process of diffusion. Many look upon it as an ion exchange reaction in which, for example, the bicarbonate ion within the cell passes outward and exchanges for an incoming nitrate or chloride ion. Jacobs (150) has shown that the HCO_3 ion can very readily pass through the cell walls, especially where the pH of the outside solution is greater than inside the cells. In a similar way H^+ ion formed inside the cells would exchange for cations. The H^+ ion also has a very high rate of transfer.

In saline soils, which are usually calcareous, the pH value of the soil solution at normal moisture contents is about 7.0 to 8.0. In such an environment, according to the ideas of Jacobs, HCO_3 would rapidly pass out from the root cells permitting other anions to enter.

Steward and Preston (317), working with potato discs in potassium and calcium salts ranging from 0.75 to 75 milliequivalents per liter, concluded that the cations were the effective ions of the salts. Potassium salts acted conversely to calcium salts for most processes; thus potassium salts increased respiration whereas calcium salts with the same common anion depressed the process.

Lundegarth (201) does not agree with the theories of Hoagland and Steward concerning salt absorption. He claims that respiration is closely correlated with anion uptake.

The unequal rate of entry of various salts is dependent in part on

the rate at which one of the ions can be transported upward into the shoot. Of interest in this regard is the observation that in bean plants (77) sodium accumulated in the roots and was not transported upward, while in sugar beet, a salt-tolerant plant, sodium passed upward into the leaves in large amounts (56). Collander (41) has reported that halophytes take up large quantities of sodium, while *Fagopyrum esculentum, Zea Mays* and *Helianthus annuus* were distinguished by their large exclusion of Na.

Some plant species, such as those of *Statice, Frankenia, Tamarix* (117, 160, 287), *Saxifraga* (344), *Ameria* and *Plumbago* (284), have the faculty of carrying salts to the exterior of the leaves where they are precipitated and later lost by wind or rain. Other plants sometimes reduce their salt content by abscission of leaves high in salt. Thus Hayward and Long (unpublished) find the first peach leaves emerging in the spring have very high salt contents and show salt symptoms when grown on saline substrates. These leaves drop early; later leaves are more normal with lower salt contents and no visible symptoms of salt injury.

Antagonism

Many writers, particularly Osterhout (41, 97, 159, 240, 253, 254, 294), have published results indicating that plant growth is reduced by unfavorable proportions of ions. No attempt will be made to review the literature on antagonism. Thus Miyake (240) states: "potassium and magnesium or calcium salts are poisonous to the rice plant when used separately but when mixed together in suitable proportions the poisonous effect more or less completely disappears". He also indicated that antagonism between cations was greater than between anions, but several have felt that antagonisms between anions should not be dismissed (269, 317). This theory as to how alkali salts affect plants, dominant up to about 1930, has subsided because of tests which show the close relationship of growth depression to osmotic pressure, and that merely concentration of a favorable physiologically balanced solution produces reduced growth (110, 247, 294), while at high concentrations growth is reduced, no matter what the ionic proportion may be (159, 294) .

A particular case of physiological balance, that of the calcium-magnesium ratio, has received much attention, particularly in nutrient and sand culture solutions. It is believed that under certain

saline soil conditions unfavorable ratios may exist and reduce plant growth (194, 271).

The early investigators of salinity conditions noted that magnesium salts and sodium carbonate were particularly toxic (99, 308). Later reviews have been made by Miller (238) and Maximov (220). The early papers stressed that the best Ca:Mg ratios were about 2:1 or 1:1, although Gile (87) gives interesting data on rice in which high Ca:Mg ratios were toxic, especially in high concentration. Martin (218) states ratios of 1:1 or 1:3 are nontoxic to rice.

Magnesium is a constituent of chlorophyll, and calcium enters into the formation of the cell wall (44, 275, 314). With an excess of magnesium it is held that a normal calcium pectinate of the proper colloidal properties is not formed in the cell walls.

In soils, examples of unfavorable calcium:magnesium balance are rare. The normal soil is principally calcium saturated (130, 139), and calcium usually exceeds magnesium in the soil solution. Yet where the soil solution is obtained at low moisture contents, cases where magnesium exceeds calcium have been observed in a number of saline soils (213, soils 75, 83, 222, 86). The coastal soils of California seem to have more than normal amounts of exchangeable magnesium (167), and in some of these soils unfavorable Ca:Mg ratios may occur. Ratner (271), in reviewing Russian work, states that soils containing over 70% replaceable Mg are unfavorable to plant growth probably because calcium relations are disturbed. Tamhane (322) finds some Indian soils where magnesium salts are more toxic than sodium salts except Na_2CO_3.

Disturbed Metabolism

Although plant growth has been shown to be related to the osmotic pressure of the substrate, the mechanism of the action causing reduced growth is unknown. It has been suggested as being due to irreversible precipitation of protoplasm in cells with high osmotic pressures. Many believe that this is not directly a cause for reduced growth. Arguments are advanced that as a result of this physiological drought the normal nutrition of the plant is disturbed and reduced growth and, in some cases, death result from this disturbed nutrition. This can be discussed under several headings.

Nitrogen metabolism. pH values of western saline soils at normal moisture contents and carbon-dioxide pressures lies between 7.0 and 8.5 (71, 214, 276) and for alkali soils varies from 8.0 to 10.0. The pH value of the notorious sodium carbonate-containing soil from Vale, Oregon, is about 10.0 at field moisture (10.75 in a 1 to 5 extract (171)). This range of pH values and the nearly universal presence of calcium carbonate is believed conducive to nitrification, although it is indicated that formation of nitrates is restricted by soil conditions above pH 7.7 (39). Hence it is assumed that the greater part of the nitrogen is absorbed as nitrates in western irrigated regions.

Nightingale and Farnham (247) and Hayward and Long (110) grew plants at increasing osmotic concentrations obtained by concentrating the nutrient solution. Thus the ratios of ions was kept fairly uniform, but at the higher osmotic pressures the nitrogen supply was extraordinarily high. Both showed by anatomical examination, (247) with sweet peas up to 3 atm. and (110) with tomatoes up to 6 atm., that at high concentration the plants were in a high carbohydrate condition despite the presence of ample nitrate. Hayward and Long found that with increasing concentration the vascular bundles were reduced and parenchyma tissue increased on a relative basis with some starch accumulation. Hayward and Long also grew tomato plants with increasing osmotic pressure obtained by adding chloride or sulfate salts to a basal nutrient solution with similar results (110, 112).

Study of nitrogen and carbohydrate fractions in plants grown under varying osmotic pressures have been made (112, 247, 345, 346). The last two studies were made with beans. In the first study (247) the roots continued to absorb nitrates, but lost their ability to assimilate it at high osmotic pressures. As a result carbohydrates accumulated. In the study of Wadleigh and Gauch (346) the nitrogen level in the nutrient solutions was held constant. Their plants were grown during the winter with relatively low light conditions, and they were unable to note any increased starch accumulation at the higher osmotic levels.

In another study beans were grown in soil to which sodium chloride had been added in increasing amounts (345). At the close of the experiment the concentrations of salt in the soil solutions and the carbohydrate and nitrogen fractions of the beans were deter-

mined. In another portion of the experiment, soil moisture content was varied so that in one case there were several degrees of water stress, and in the second, several stages of physiological drought because of salt additions. In both series with increased "drought" there was an increase in nitrates and protein N, and little effect on reducing sugars. Addition of salt caused a large decrease in starch in the leaves. The authors concluded that with water stress, hydration of protoplasmic proteins was reduced, and suggested that other factors were operating to reduce growth besides water stress in the salt series, such as an effect of the chloride ion *per se*. Decrease in starch formation with wilting or high osmotic pressures are also reported (147).

Nightingale has reviewed the general subject of nitrogen metabolism (245), and Bakhuyzen (16) has reviewed and discussed protein synthesis and growth during the various growth stages of wheat. During the third, or catabolic, stages there is a breakdown of proteins and accumulation of carbohydrates similar to the accumulation of carbohydrates which occurs in high-salt plants. He shows that this is linked with reduced respiration and probably also reduced formation of auxin which may regulate respiration. Steward and Preston (317) state that potassium salts stimulate both respiration and protein synthesis from stored amino acids, while calcium salts retard the process. In calcium solutions nitrogen metabolism is diverted from amino acids to amide and ammonia. Phillis and Mason (260) suggest that potassium controls the rate of photosynthesis by regulating the rate at which carbon dioxide diffuses to the chloroplasts and the rate of movement of sugar from the chloroplast to the phloem. It is known that stomatal openings are reduced in cases of moisture stress (67, 68, 293).

Crops like sugar beet which normally store sugar will have a lower sugar content in the presence of high nitrates, even though this is accompanied by high salt substrate (115). Relationships between carbohydrate supply and absorption have recently been reviewed (139).

Other metabolic and specific ion effects. While there is overwhelming evidence that reduced plant growth in saline substrates is correlated with osmotic pressures, specific effects of certain ions on plant growth also exists.

The halophyte *Salicornia* had the highest germination and growth

in a soil mixture containing about 2% NaCl (334). It seemed to require the Na and Cl ions, and other salts with equal osmotic values were far less satisfactory.

Among agricultural plants effects associated with certain ions have often been noted in experiments in which comparisons were made at equal osmotic pressures. Chloride salts have frequently been found to be more toxic than sulfates. Thus Hayward and Long (111) reported death of peach trees at 3.2 atmospheres with chlorides, whereas trees were still alive with sulfates at 3.7 atmospheres. These trees ultimately died. Similar results were obtained with alfalfa (209).

On plotting yields against freezing point depressions from the data of Eaton (56), sulfates are found to be more toxic than chlorides at equal osmotic levels for alfalfa, cotton and sugar beets. Sulfates were found to be more toxic than chlorides for flax (114) and tomatoes (110).

While bicarbonates occur in large proportions in some irrigation waters, they are usually present in minor proportions in saline soil solutions. It is very difficult to determine whether the bicarbonate ion by itself is toxic because of the pH and carbonate ion relationships. Steward and Preston (316) found that potassium bicarbonate and dissolved carbon dioxide at constant pH, both reduce protein synthesis. In a three-dimensional diagram they show that protein synthesis in potato discs is at its maximum at about pH 6.5 and that concentrations of $KHCO_3$ above 5 m.e. per liter result in decreased protein synthesis. At 20 m.e. per liter protein synthesis is almost completely suppressed. The Arizona investigators (30, 229) feel that additional carbon dioxide in their soils would improve them by lowering the pH value and increasing phosphate availability.

In addition to this the cation associated with the bicarbonate ion is of extreme importance; if sodium it may lead to formation of an alkali soil, while if calcium it may be precipitated as $CaCO_3$.

The effect of cations on plant growth has been extensively investigated in various tests on antagonism. When calcium, magnesium and sodium are added singly to an otherwise adequate nutrient solution or sand culture, magnesium is more toxic than the other two (209, 347, 350). In part, this is attributed to unfavorable Ca:Mg ratios, or the phenomenon of antagonism which has been discussed previously. The very striking toxicity symptoms with guayule

(347) indicate that this plant is particularly sensitive to excessive magnesium. Calcium chloride was more toxic to barley than sodium chloride when compared on an equal osmotic basis (47). In experiments with flax on a podsol soil Smirnov (311) reported calcium to cause the greatest decrease in yield as compared with sodium and potassium. All were added as chlorides to produce equal osmotic levels.

Many writers have attributed the unfavorable effects of alkali salts to the sodium and chloride ions (273, 274). Repeated tests in which sodium chloride has been compared with calcium chloride has failed to show that it is unduly toxic in sand and solution cultures at isosmotic concentrations (75, 76, 77, 112, 114, 209, 216, 311, 319, 331, 347). In small amounts sodium is sometimes found beneficial to growth of sugar beets (191, 192) and asparagus (283). Sodium is accumulated in the roots of beans when grown in solutions of high sodium content (75), and this has also been found true for peaches (unpublished).

That chloride fertilizers may have some specific effect on reduction of chlorophyll has been suggested (19) and that as a result starch production is reduced (18). Breazeale (27) suggests that the alkali salts destroy the enzymes in the roots.

Germination and Seedling Growth

One of the processes associated with germination is imbibition which is governed by osmotic forces. It is natural, therefore, that imbibition should be retarded in salt solutions.

The Wyoming investigators as early as 1898 found that water imbibition and germination of the seeds used varied with the osmotic pressure of the salt solution (35, 36, 309, 310). Germination of wheat was prevented at about 10 to 14 atmospheres, while rye tolerated slightly larger amounts. Russian workers as early as 1912 (331) reported that germination of seeds was correlated with the osmotic pressure of the soil solution and found that isotonic salts gave approximately the same results (186).

Rudolfs (282) tested the germination of wheat, corn, watermelon, Canada field peas, white lupine, soybeans, rye and alfalfa at concentrations of various salts up to seven atmospheres. He concluded that "the rates of absorption are progressively retarded with an increase in the osmotic concentration values of the solutions which do

not react with the seed substance to modify in a chemical way the imbibing properties of the seeds" (109, 295, 330).

Hicks, working with fertilizer salts (124a), concluded that "the chief injury to germination from chemical fertilizers is inflicted upon the young sprouts after they leave the seed coat and before they emerge from the soil". Unpublished data (109) on wheat and alfalfa are in agreement with the above. Many other investigators (99a, 99b, 99c) using soils were unable to agree with the Wyoming workers, which can now be explained by the fact that through reaction with the soil the salt composition and concentration was changed, and that they were dealing with both emergence of the plumule from the seed and early growth of the seedling, or that they were working with alkali soils of low salinity and high content of exchangeable sodium.

Working with wheat seedlings in solutions, Harter (108a) found half of them died at normalities given: sodium chloride 0.05, sodium sulfate 0.04, sodium bicarbonate 0.026, sodium carbonate 0.01, magnesium chloride 0.009, and magnesium sulfate 0.007. This range corresponds to 0.8 atm. to 0.2 atm. These values seem exceptionally low. When mixed, or with calcium sulfate, the seedlings could tolerate much larger quantities, up to 0.2 N (about four atmospheres) except where sodium carbonate was present (162). Magnesium chloride alone was more toxic than sodium or calcium chlorides (215).

The need for oxygen during germination and seedling growth was shown in reports (343) that barley root and shoot growth was considerably diminished at pressures below 9.5% oxygen, while rice grew well at oxygen pressures as low as 3%. Temperature is also an important factor in germination and early growth. Thus Ogasa (250) found the maximum concentration for germination of soybeans to be 0.3 N NaCl at 15° C. and only 0.2 N NaCl at 30° C. Edwards (59) reported the optimum temperature for soybean germination to be 33–36.5° C., while 28–31° C. yielded the longest seedlings. Ahi and Powers (2) found the best temperature for germination to be 55° F., and at this temperature strawberry clover would tolerate 5,600 p.p.m. of sea salts. Alfalfa withstood somewhat less.

Fertilizer injury to seeds and reduction in stand is also an osmotic phenomenon similar to the action of alkali salts (270, 330). It thus

appears that seeds will germinate (*i.e.*, plumule emerge) at higher osmotic pressures than the shoot will tolerate later in its growth period, but germination at high osmotic pressures retards germination and probably results in a weakened radicle and plumule.

PLANT RELATIONS TO ALKALI SOILS

As indicated previously alkali soils are those which contain considerable replaceable sodium. On reduction of the electrolyte content in such soils the replaceable sodium will hydrolyze to give soil pH values which are usually above 8.5, and the soil will disperse.

Plants growing in alkali soils having a high salt content have the same ills as those in saline soils and in addition may suffer from actual lack of water, lack of oxygen in the soil air, and many nutritional disorders as a result of impermeability to water, plant food unavailability, and high pH values.

A number of investigators (11, 28, 30, 124, 128, 229, 255, 329) have attributed poor plant growth on alkali soils in large part to the unfavorable physical conditions in such soils, and because many alkali soils are dispersed or puddled, Arizona investigators have made a study of plant growth conditions in puddled soils (226, 231).

Puri (265) reported studies relating yield of wheat to degree of alkalisation on two soils in the Punjab. His degree of alkalisation is the per cent saturation of the soil complex by Na + K. He reported that, on the Montgomery soil, yields increased from 32 pounds per acre with 67% saturation to over 1,000 pounds per acre with the replaceable sodium reduced to about 20% on soils treated with calcium chloride and leached. Similar results were obtained with a Kala Shak Kaku soil, the correlation coefficient between degree of alkalisation and yield for the two soils treated separately being 0.85. Similar results were obtained elsewhere (362). Replaceable magnesium in excess of 19% of capacity augments the depressing effect of replaceable sodium on crop yields (252). Ratner (271, 272) grew oats and wheat in a chernozem soil saturated with sodium to varying percentages. In pot cultures growth was good up to about 50% sodium. Naturally occuring solonetz soils in pots planted to oats, wheat and barley gave lower yields with 30% replaceable sodium than with 10% replaceable sodium. Presumably in these pots aeration was good. Gedroix (84) planted oats, and in some cases buckwheat and mustard in soils saturated

with the various bases including sodium. He did not obtain normal growth unless exchangeable calcium accounted for a major portion of the exchange capacity, although strontium-saturated soils gave fair yields.

Soils of Low Permeability

Alkali soils containing a considerable amount of soluble salts (saline alkali soils) are usually permeable because the salts present maintain the soil colloids in a flocculated condition. As the soluble salt concentration is reduced by irrigation water the flocculative effect of the soil solution is reduced, soil structure is broken down, the clay disperses and the finely divided clay particles thus separated fill up the finer capillaries and reduce or prevent movement of water and air. The effect of electrolytes on soil floccculation can best be explained on the basis of zeta potential (157). They found that sodium-saturated soils have high zeta potentials, and it requires two to ten times as much of sodium salts in solution as on the colloid to reduce the zeta potential below a critical value and so produce flocculation. Calcium-saturated soils have a much lower zeta potential, and far less calcium salts in solution are required to produce flocculation. As an example of this, Reitemeier and Christiansen (unpublished), working with an Indio fine sandy loam, one part of soil to two of water, found 12 m.e./liter of a calcium salt was necessary to produce flocculation with a 25% Na-saturated soil, while only 2 m.e./liter was sufficient with a 10% Na-saturated soil.

Other theories concerning the cause of impermeability in soils have been prepared. Scofield (289) has suggested that on leaching out salts of alkali lands, sodium and other soluble silicates are precipitated in the soil closing the capillaries. Kelley and Brown (171, 232, p. 265) have shown that soluble sodium silicate can be found in some alkali soils. Arizona investigators (232) and Kowda (187) also suggest that aluminum hydroxide is present and may be precipitated in the pore spaces.

In a comprehensive study of alkali soils Gardner (71) found that permeability decreased with clay content and exchangeable sodium content, but that replacement of the sodium by calcium did not result in increased permeability unless the soil was first dried.

Men at the Punjab Irrigation Research Institute have recognized that dispersion of alkali soils is one of the major reasons for poor plant growth. The degree of dispersion (in per cent of complete

dispersion of all the clay and called "dispersion coefficient") is governed largely by the percentage of replaceable sodium (10, 61, 64, 98, 134, 184, 185, 189a, 242, 263, 267, 290, 355), but modified too by salt content (14, 61, 266, 323). Ratner (272) and Kotzman (185) claim that even as little as 5% replaceable sodium increases dispersion, while one author (14) indicates that soils containing as little as 3% Na may have some alkali soil properties. Of interest is the observation that when rice is grown in alkali soils over pH 8.8, soluble salts may be beneficial because of the flocculative action on soil colloids (234).

Working with electrodialysed acid clays, Bradley, Grim and Clark (26) showed that as the montmorillonite clay crystal took on additional water, it swelled, and as the water content increased to 65%, corresponding to 24 molecules of water per unit cell, no distinct X-ray patterns could be obtained, thus indicating that the plates forming the soil crystal were free and probably dispersed. These dispersed colloidal platelets, previously forming a crystal, can move about in the soil solution and may close the soil capillaries.

As irrigation water removes the accumulated salts, the soil will disperse when the salt concentration is reduced below certain critical limits. This is often called "freezing up", and at this stage permeabilities (71, 212) may be as low as a few inches a year. A good growing crop in the Southwest such as alfalfa may require 60 inches of water per year. Clearly, if the rate with which water can enter the soil is less than 60 inches a year, this plant can not get enough water for maximum growth even though the soil is covered with water the year round. The plant then, on some heavy alkali soils, can suffer from lack of soil water as well as lack of soil oxygen and other causes.

Many crops grown in heavy soils of humid regions have been found to suffer from lack of oxygen in the soil air. In alkali soils having low permeabilities to water it is very likely that the soil air is deficient in oxygen. There seem to be very few data on this subject from alkali soils.

Nutrient Unavailability and Chlorosis

Chlorosis is very prevalent on irrigated land throughout the West and particularly in the northwestern States. It is usually associated with calcium carbonate in the soil at a depth of less than three

feet. The crops affected most frequently are grapes, pears, soft maple, cottonwood, brambles, peaches, roses, apples, prunes, globe locust and citrus (20, 315, 354). This chlorosis is considered lime-induced because applications of iron as sprays or injections give some benefit, but often not enough to be economical (20, 193). In some localities zinc and boron deficiency are also present. Guest (96), in a study of citrus nutrition, reported that citrus grew chlorotic at pH 7.0 in the presence of 1% coarse magnetite but grew well at the same pH value if the magnetite was finely ground. He attributed the increased growth with finely ground magnetite to greater root-contact surface and absorption of iron. This suggests that in calcareous soils trees may become chlorotic because the area of contact between roots and iron-containing soil minerals is decreased to a low value, and that as regards iron at least, contact feeding may be relatively important (153, 155).

   Soil reaction. Whitney and Gardner (361) have recently studied the effect of carbon dioxide on soil reaction. They find that the pH value is approximately a straight line function of the logarithm of the carbon dioxide pressure. Thus for a black alkali soil (Gila series), pH values at 1:2 suspension varied from 9.20 at 0.003 atmosphere carbon dioxide to 7.06 at 0.1126 atmosphere carbon dioxide. The carbon dioxide content of the soil air in the vicinity of the root may be very low unless the plant is growing vigorously. In soils which do not contain appreciable absorbed sodium or sodium carbonate the pH values at carbon dioxide contents up to 20% would probably range from 8.2 to 6.5. The pH value of a soil varies with its content of $CaCO_3$, $MgCO_3$, Na_2CO_3, replaceable base status, carbon dioxide pressure, salt content and dilution.

   Mehta in the Punjab (234) has correlated rice yields with pH and soluble salt content. Above pH 8.8 yields decrease, likewise if the salt content exceeds 0.24%. Since the rice was flooded this salt concentration in atmospheres was probably low. The soil pH values are related to the degree of sodium saturation (265), and it is inferred that the soils are unproductive at high pH values because of the poor physical condition. When the soil pH is as low as 8.8 the growth of other crops less tolerant than rice can be considered (234).

   Plants grown on soils containing calcium carbonate often have saps of high pH values which reduce iron solubility and mobility

(196, 197). Plant sap pH value and soluble iron content also varies with light intensity (148, 197) and nitrate level (348).

Calcium. McGeorge (225) has suggested that in the soil solution of alkali soils the carbon dioxide content and calcium ion content are low. This is also indicated by analyses of chlorotic leaves of trees grown on areas which indicate a low calcium content and probably low calcium availability (227). Further evidence of low quantities of soluble calcium is given by negative calcium absorption in Neubauer tests on such soils (228). Magistad and Reitemeier (213), in their analyses of soil solutions at low soil moisture contents, reported two cases in which the calcium content was 6.4 and 6.9 milliequivalents per liter. This quantity continuously supplied is considered more than ample for plant growth, but there may be other soils which are more deficient in soluble calcium or in which the supplying power of the soil is meager. Ratner (271, 272) also concludes that plants suffer for lack of calcium in high sodium soils and states: "The destruction of plant life in pot experiments when there is a large amount of exchangeable Na is difficult to explain (in the case of non-carbonate soils) by the alkaline reaction of the medium, by the accumulation of soda, or by the unfavorable physical properties of the soil. One of the possible causes of the destruction of plant life may be assumed to be the breaking down in the soil of the calcium regime, and in particular an insufficiency of calcium as one of the elements of plant nutrition". A similar concept has been expressed elsewhere (11).

An excellent review of the role of calcium for higher plants was published in 1938 (183), listing three hypotheses on the action of calcium on plant roots:

a. Loew's: That the cell nucleus requires specific ions such as calcium.

b. Kisser's: That a lack of calcium results in disintegration of the cell wall.

c. Hansteen-Cranner hypothesis: That single ions can act as toxic agents on the cell wall or prevent such toxic action.

This author also discusses a number of articles indicating that in plants calcium has a regulating action on the water content, modifies carbohydrate and nitrogen transformations, respiration, enzymes, germination and laying down of tissue. It also forms calcium pectates in the middle lamella having desirable properties. See also (246).

There appears to be some similarity between strongly acid soils and soils high in replaceable sodium or magnesium, in that in each case the plant may be unable to obtain an adequate supply of calcium. Albrecht and his students (4, 5, 6, 7, 92, 144, 264) and others (84, 86, 208, 233, 261, 333) have indicated that plants suffer for lack of calcium in acid soils rather than because of the H-ion concentration. Alkali soils which have a low content of replaceable calcium and conversely a high percentage of replaceable sodium or magnesium are also believed to reduce plant growth because of calcium deficiency. The work of Jenny and associates (152, 154, 155) has demonstrated that in contact with clays, plant roots may lose large amounts of ions to the clay by contact depletion. Of particular interest are the experiments of Van Itallie (149) who grew Italian rye grass in soils saturated principally with calcium, magnesium, sodium and potassium, respectively. The amount of cation in the plant correlated with the percentage of that cation on the exchange complex. Thus soils low in exchangeable calcium and magnesium, and high in sodium, produced a crop having a high Na content, and growth was somewhat reduced.

In studies on germination of barley in alkali soils Kelley (164) reported that the soil solution in contact with the seeds did not materially reduce germination but that the seeds in contact with the alkali soil failed to germinate properly. He believed this to be caused by the avidity of the soil complex for calcium, even to the point of removing calcium from the barley seed and roots. Similar experiments in which "bara" soils, freed from salts by washing, still prevent germination are reported (108). The author explains the phenomenon on the basis of formation of black alkali in the absence of soluble salts, but Kelley's hypothesis seems more plausible. In harmony with this concept, Tiedjens and Schermerhorn (326), in discussing greenhouse problems, state: "The ratio of available calcium to potassium or sodium was extremely important for germination of seed and growth of vegetable crops on coastal plain soils".

Russian workers (252, 272) indicate that plant growth is not seriously affected in non-saline alkali (solonetz) soils until the percentage of replaceable sodium exceeds 40. Their experiments were conducted in pots carefully watered so the unfavorable physical effects of sodium were minimized. They also attribute lack of growth at high replaceable sodium percentages to lack of calcium

and report that replaceable magnesium lowers the threshold of sodium toxicity, as also does organic matter and calcium and magnesium carbonates. Ratner (272) suggests that calcium and magnesium carbonates may increase the unfavorable effect of sodium because of the formation of small amounts of sodium carbonate in the soil. The harmfulness of organic matter is attributed to increased alkalinity due to hydrolysis of Na-humate, to direct toxicity of Na-humate, and to additional mobilization of calcium. (Authors probably meant immobilization (252)).

Chlorosis of trees on calcareous and alkali-calcareous soils may possibly be caused by a low calcium supply, since the leaves of such trees often have a high potassium and low calcium content (193, 225, 352, 353). In experiments with alfalfa in soils of various Ca/K ratios, the Ca/K ratio in the plant was found to correlate with that in the soil (145). Peech and Bradfield (257) have recently reviewed calcium-potassium relationships in soils and plants and conclude that plants absorb large amounts of potassium because large amounts are available and that large amounts of available potassium decrease calcium absorption. Alkali soils often have several milliequivalents of replaceable potassium per 100 grams, far more than humid soils, and the low calcium content of chlorotic leaves may therefore be associated with an unfavorable K/Ca balance in the soil solution. Reference to the table on page 183 shows that alkali soils tend to have a higher relative proportion of replaceable potassium.

Magnesium. There appears to be no evidence of magnesium deficiency in alkali soils. Supranormal amounts of replaceable magnesium leads to unfavorable calcium-magnesium relations. Ratner (271), in reviewing this, states that the experiences of several Russian investigators have shown that with magnesium up to 50–60% of the replaceable capacity there is no decrease in yield, but above this there is a sharp decrease in plant growth. He interprets this as additional evidence that conditions such as high Na or Mg will limit calcium availability and intake by the plant and thus reduce growth. Several soil analyses from coastal California are listed (167) in which magnesium constitutes over 50% of the base exchange capacity. McIntire and Young (202) grew tall oat grass and cowpeas in soils to which MgO and $MgCO_3$ had been added. During the first year germination was poor, chlorosis appeared and

yields were low. At the end of a year this toxicity had disappeared. When the soils were leached at the time of harvesting cowpeas, magnesium salts greatly exceeded calcium in the leachate.

Iron and manganese. Shive (296) has indicated that the familiar lime-induced chlorosis is a disturbance of the soluble iron-manganese ratio in the plant. Thus chlorosis might be caused by iron being too low and remedied by applications of iron as a spray, by tree injections or soil applications. Sometimes manganese is too low, and such cases are remedied by manganese applications (217).

As Thorne and Wallace (325) have pointed out, chlorosis on high-lime soils (and almost all alkali soils are high in lime) is generally due to a deficiency of either iron or manganese in the plant. They indicate that ferrous iron can not exist in appreciable quantities in normal calcareous soil. Ferrous iron availability then depends on the reducing ability of the root. There may also be a disturbance of ferric-ferrous relations within the plant.

Anions. The anions essential to the growth of plants are usually abundant in the soil solutions of alkali soils, with the exception of nitrates and phosphates. Breazeale and McGeorge (30, 230) state that few, if any, black alkali soils contain sufficient black alkali to be directly toxic to plant growth, but that the plants are unable to absorb the necessary plant foods, including nitrates and phosphates, at pH values above about 8.0. In well aerated soils the root can grow and excrete sufficient carbon dioxide to reduce the pH value of the local root environment so as to facilitate plant food absorption, primarily that of phosphates. Addition of organic matter which yields carbon dioxide improves plant growth on alkali soils.

Buehrer (34) has calculated the relative concentrations of the various phosphate ions at pH ranges from 3 to 10, and shows that on the acid side HPO_4^{--} is the prevailing ion. In alkaline solutions it is $H_2PO_4^{--}$. While chemical analyses show an ample supply of soluble phosphorus in soil solutions of alkali soils, this phosphorus is presumably present as $H_2PO_4^{--}$ and the plant does not seem to absorb it readily. On such soils phosphate fertilization is often beneficial (30).

Absorption of nitrate ions is not markedly retarded at pH values up to 8.0 (13, 140, 247), but its intake is governed by the rate with which it is removed from the root cells and transported to the rest of the plant. In the field, nitrate absorption must be satisfactory in

calcareous soils because excellent crop yields are often obtained. Nitrate formation is greatly reduced in alkali soils and probably does not occur above pH 7.7 (39).

Corrosive and Toxic Substances

Workers since the time of Hilgard in 1886 (125) have noted that sodium carbonate in soils is actually corrosive to plants, usually at the soil surface level or crown of the plant (125, 143, 358). Many of the older soil analyses report more sodium carbonate than was actually present, but there is no question that many alkali soils contain substantial amounts of sodium carbonate as such.

Magistad (206) found that at pH values above 9.0 appreciable amounts of aluminum were in solution as aluminates and suggested that very alkaline soils do not support plant growth because of the soluble aluminates present. Some salty soils from the humid climate of Finland contain a high content of alum (1), and others (187, 232) also consider that aluminates are present in some alkali soils.

Plant Tolerance to Salty Soils

The literature on this subject is profuse, and it is impossible to review it in this article. Most of the data on tolerance fails to take into consideration the distinction between saline and alkali soils, and the effect on the plant may be quite diverse. Furthermore, most of the data is given in terms of per cent salt in the soil without reference to water content, so that osmotic pressures in the soil solution can not be calculated (163, 200).

Peaches are among the crops least tolerant to salinity conditions, dying at about 3.0 atmospheres (111). On the other hand, alfalfa and cotton are among the most tolerant and are reduced in yield about 20% at osmotic presures of 4.0 atmospheres (210).

Tolerance to high osmotic conditions may not carry with it tolerance to alkali conditions, particularly high percentages of replaceable sodium. Thus it is suggested (252) that peas, because of their high calcium requirement, will not tolerate as much replaceable sodium as wheat. The experiments confirmed this.

SUMMARY

About 350 papers are reviewed on the subject of plant relations in saline and alkali soils. The fields stressed are: the classification

and composition of saline and alkali soils and the physiological responses of plants grown in soils of these two types. Papers dealing with reclamation of alkali land, cultural practices, tolerance of various plant species to salinity and alkali conditions, and ecological relationships are omitted.

A distinction is made between saline and alkali soils in accordance with the classification of such soils by de Sigmond. Saline soils have high salt contents—at least 0.1% in the case of chloride salts and 0.2% where sulfate salts predominate. Alkali soils contain at least 12% of exchangeable sodium plus potassium in terms of the exchange capacity of the soil. The threshold of 12% is taken provisionally because soils containing this amount of exchangeable alkali bases have impaired physical characteristics. There are gradations and combinations of saline and alkali soils so that four main classes are recognized: saline soils, saline alkali soils, slightly saline alkali soils and degraded alkali soils (alkali soils with a very low salt content).

In saline soils the principal factor depressing plant growth is the decrease in available water due to the high osmotic pressure of the soil solution. Of interest in this connection are the data of Rosene, and Hayward and Spurr which show by direct measurement that water absorption is reduced as the osmotic pressure of the substrate is increased. Some investigators attribute decreased growth in saline soils to the harmful effects of specific ions. Such effects are known to exist, but in most cases they appear to be less important than the inhibition resulting from high osmotic pressure.

Many reasons are listed why a reduction in water intake decreases plant growth. These include salting out of cellular proteins, shrinkage of cell contents from cell wall, irreversibility of hydration of cell contents and interference with ion accumulation.

Plants require considerable oxygen in the substrate for absorption of water and minerals. Many alkali soils are water-logged with low oxygen contents in the soil air, and this lack of oxygen is believed to reduce plant growth.

Plants growing in saline soils are subjected to high concentrations of certain salts and to salts which are not in the most favorable ratios for plant growth. Under such conditions several authors have found that the nitrogen compounds are not assimilated, carbohydrates accumulate and the growth rate is reduced. On the other

hand, it has been shown that starch formation may also be inhibited by high chloride concentrations.

In alkali soils having large contents of replaceable sodium, the soil takes on unfavorable physical properties. It disperses, does not drain well, the soil air may be low in oxygen, and the soils do not "take" irrigation water. The presence of considerable amounts of salts or electrolytes in soils overbalances or minimizes the unfavorable effect of exchangeable sodium on the dispersion of soils. When such salts are removed from a saline alkali soil the unfavorable physical effects are accentuated.

A number of authors ascribe the unfavorable effects of alkali soils on plants to a breakdown of the calcium regime in addition to unfavorable physical conditions. The soil solution in equilibrium with alkali soils is very low in calcium and high in sodium.

In addition to unfavorable physical condition and lack of calcium, alkali soils usually have high pH values, with attendant unavailability of several essential elements such as iron, manganese, phosphates, and at times nitrates.

In alkali soils of low salt content some sodium carbonate is present. The alkali carbonates are very toxic and even corrosive to plant parts. Probably only in the most extreme cases is there any appreciable amount of free sodium carbonate.

Soil solutions of high osmotic pressures are not confined to the saline soils of the West, but may also occur as a result of heavy applications of soluble fertilizer, especially on sandy soils.

A distinction is made between the characteristics of saline and alkali soils, since it is believed that the physiological reactions of the plant are different under the two sets of conditions. It follows that a plant may be tolerant to one soil type and not to the other.

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THE BOTANICAL REVIEW

THE STRUCTURE OF PROTOPLASM. II[1]

WILLIAM SEIFRIZ
University of Pennsylvania

INTRODUCTION

Ten years have elapsed since the structure of protoplasm was
reviewed in this journal. Five of these years have been trying ones
for those foreign countries which in the past have contributed so
much to theoretical research. The paucity in original theoretical
work has, however, been compensated for by numerous interesting
compendia of research in physical and chemical biology. These sur-
veys have appeared as textbooks, monographs, symposia, handbooks
and reports in biology and medicine. Such publications are in
themselves reviews, but they include new and original work, espe-
cially in the realm of interpretation. Among them are the large
collection of papers entitled "Medical Physics", edited by Glasser
(22); the publication in honor of Professor Bensley, edited by
Hoerr (25); the symposium on "The Cell and Protoplasm", held
at Leland Stanford University and edited by Taylor (79); the text
by Pfeiffer (59) on "Experimentelle Cytologie"; Guilliermond's
(24) "Cytoplasm of the Plant Cell"; the fifth volume of "Colloid
Chemistry, Theory and Methods, Biology and Medicine", edited by
J. Alexander (1); and the symposium on "The Structure of Proto-
plasm", edited by Seifriz (71).

Should we carry the subject of this review to its logical conclu-
sion, it would include everything that goes on in protoplasm, for
in the last analysis structure underlies all natural phenomena. It
would seem, for example, that photosynthesis and respiration could
be discussed to the fullest extent without mention of structure, yet
we could hardly escape a consideration of molecular structure, and
if we stop short of it, this is only because of the limitations of our
knowledge. Photosynthesis and respiration will not be considered

[1] Supplement to article in The Botanical Review 1: 18–36. 1935.

in this review, but such protoplasmic properties as permeability, anesthesia and frost resistance will be, for certain hypotheses of these phenomena are in learge measure based on structure.

There are a number of properties of protoplasm which appear to have little to do with structure, though actually very closely associated with it and often dependent upon it. Sometimes they are the best indications that we have of a specific type of structure, a structure which is not visible and which therefore must be interpreted on the basis of known properties. As an illustration, an elastic soap may be compared with an inelastic one. Familiarity with jellies and pastes leads to the obvious conclusion that the structural units of the elastic soap are of such a form and arrangement as to endow the dispersion with elastic properties, whereas neither the shape nor orientation of the structural units of the inelastic paste contributes to elastic qualities.

Those properties of protoplasm which are related to structure shall first hold our attention.

PROPERTIES RELATED TO STRUCTURE

Viscosity. Viscosity, in the pure and strict sense, has far less bearing on structure than anomalous viscosity, or so-called non-Newtonian behavior which is a kind of lawless flow. But before taking up anomalous viscosity something should be said on the misuse which has been made of viscosity in interpreting protoplasmic behavior.

Changes in viscosity, and in surface tension and permeability as well, though significant properties of living matter which come into play in many vital processes, are not always, if indeed usually, the cause of the phenomena which they accompany; they are more often the result of, or incidental to the processes, of which they are sometimes said to be the cause. To cite an example: when protoplasmic flow is diminished or stopped by an experimental condition, the assumption is frequently made that an increase in viscosity has occurred and that this increase is responsible for the decreased flow. The rate of protoplasmic movement depends upon two major factors, viscosity and motive force. One or the other or both may be responsible for a change in rate of streaming. The situation may be compared to water flowing through a pipe under pressure exerted by a pump. If the pump breaks down and flow stops, the

viscosity of the water has not therefore increased. Kamiya (26) has found it possible to stop protoplasmic streaming by mechanical pressure without any change in viscosity occurring. Similarly have surface tension and permeability been resorted to in explanation of changes in cellular activities. They are undoubtedly involved and may at times be the cause, but they are just as likely to be among the consequences. If a cell is in a pathological condition from any cause, its control over selective permeability is probably lost to some degree, but the change in permeability is not therefore the cause of the pathological condition. One may make another analogy: diabetic patients have acid blood, but diabetes is due to a breaking down in sugar metabolism and not to the loss of acidity control.

Viscosity changes are significant in cellular physiology. They are best described as diagnostic symptoms. They are sometimes closely correlated with structural changes, as in thixotropic setting and collapse; but it is the anomalous flow of liquids which so closely bears on structure and is so important an indicator of a specific type of structure.

Anomalous Viscosity. When a pure liquid, such as water or glycerine, or a true solution flows it obeys Newton's law, $v = \phi \, Fr$, in which v is the velocity of flow, ϕ the fluidity, and F the shearing stress on either of two planes separated from each other by the distance r. If the flow fails to follow Newton's law, then something can be said about structure. It is theoretically to be expected and experimentally capable of demonstration that non-Newtonian substances, those which in solution do not exhibit true viscous flow and therefore do not follow Newton's law nor the formula of Poiseuille, possess structural features not present in Newtonian substances. The best illustration of this is that of two soaps, presumably of identical chemical constitution, both sodium stearate, but one Newtonian and the other not. Colloidal dispersions of these soaps, apparently identical, were not so in physical qualities; one exhibited true viscous flow whereas the other showed variable viscosity, the viscosity value varying with each pressure applied. The former Newtonian soap had a granular amorphous structure, microscopically viewed; the latter was crystalline in nature, its dispersed particles were slender fibers, hence the anomalous behavior. Furthermore, the anomalous soap was elastic and the Newtonian soap inelastic.

The distinguishing features of non-Newtonian solutions are two: with each change in pressure they yield a different viscosity value until a definite maximum pressure is reached, at which point the viscosity value remains constant; and they require an initial pressure to start flow; this pressure is termed the yield value. The Society of Rheology owes its origin to these misbehaving non-Newtonian solutions (12). Out of that Society much good has come, but with it one confused issue, the introduction of the term "plastic" to indicate the general character of non-Newtonian substances. This is an unfortunate choice of words, for plastic substances maintain any form into which they are molded, whereas elastic substances do not, and non-Newtonian substances are elastic. Putty is plastic, gelatine not, and gelatine is elastic; solutions of gelatine are non-Newtonian.

Experimental proof of the non-Newtonian character of protoplasm is very meager. A decade ago one could have questioned it, but on experimental grounds only, not on theoretical grounds. Now, however, owing to the work of Pfeiffer (58), it can be conclusively stated that what was before theoretically evident, is now experimentally proved. Pfeiffer has shown that if protoplasts are deformed under different pressures, curves showing pronounced anomalous viscosity are obtained. The flow of protoplasm is therefore not obedient to the laws of Newton, Poiseuille, and Stokes. That protoplasm belongs to the large group of organic substances which are non-Newtonian in behavior has been repeatedly emphasized by Seifriz (66). Needham et al. (48) use anomalous viscosity and related properties as a basis for classification of proteins.

Northen and his collaborators (52–55) have carried out a series of studies on structural viscosity which indicate the bearing of non-Newtonian qualities on protoplasmic structure. In order to explain some features of stimulation, Northen (52) advanced the hypothesis that stimulation breaks down the protoplasmic network. He adds that many features of irritability may be explained if protoplasm is considered a reversibly dissociable system. Northen and MacVicar (55) found that when filaments of Spirogyra are subjected to X-ray dosages from 250 to 5000 r units, the structural viscosity at first decreases, and then, with longer exposure, increases. Decrease in structural viscosity may indicate that constituents of the protoplasmic network have dissociated or that long protein mole-

cules have shortened, whereas increases in viscosity would mean that molecules have extended or that reassociations have occurred. Muir (45) applies the same interpretations to changes in viscosity due to salts and oleates. He says that decrease in structural viscosity brought about by these substances may be the result of a weakening or destruction of bonds such as those existing between the lipophilic side chains of protein molecules. Northen (52) interprets the anesthetic effects of fat-solvents in terms of readjustments in protoplasmic structure.

Double Refraction. Many of the physical properties of protoplasm are so closely correlated, so intimately interdependent, that to mention one is to imply the others. This is true of the relationship between the non-Newtonian behavior of protoplasm, its double refraction or flow birefringence, fluid crystallinity, elasticity, contractility, and continuity in structure. The double refraction of protoplasm has been frequently denied because it is not readily demonstrable; actually, however, its presence has long been known. Englemann (19), as far back as 1875, observed it in pseudopodia; later Weber (81) described it in chloroplasts, which, though not of protoplasm, are closely akin to it. And, finally, Schmidt (64) has described double refraction in *Amoeba.*

Flow Birefringence. Birefringence is synonymous with double refraction, but as flow birefringence it becomes a special case particularly applicable to protoplasm, for the living substance is often in a state of flow. Birefringence in a flowing liquid may be seen in certain colloidal suspensions such as that of vanadium pentoxide, but only when these are in a state of flow. This behavior means that linear particles, present in the liquid, are scattered in a disorderly fashion when the liquid is in a state of rest, but during flow they become oriented, and double refraction or flow birefringence results.

Flow birefringence is becoming a commonplace in biological literature. Some years ago the reviewer insisted that protoplasm must and would show double refraction if proper tests could be made. Up to that time only the forgotten work of Engelmann (19) existed in support of it in protoplasm. Later came the direct work of Schmidt (64) and the indirect evidence of Pfeiffer (58). Both indicate that fibril molecules are present in protoplasm, that these are the cause of anomalous viscosity and birefringence.

Great emphasis on birefringence in organic matter has been laid

by Needham and his co-workers (17, 29, 30, 46, 47) in their study of protein solutions. Their problem was a combined study of anomalous viscosity and flow-birefringence. Joining these two phenomena in one study indicates appreciation of their close relationship and the interdependence of all properties based on a specific type of structure. As a result of their experiments on protein solutions, both in bulk and as surface films, Needham and co-workers (29, 30) class proteins into three major groups. The first group consists of those proteins which show flow-anomaly, *i.e.*, are non-Newtonian, both in the bulk phase and in the surface film. They also show flow-birefringence in the bulk phase. Examples are tobacco mosaic virus, nucleoprotein and myosin. Though corpuscular proteins, they have elongated particles before denaturation. The second group consists of those proteins which show flow-anomaly only in the surface film and no flow-birefringence in the bulk phase. They are probably close to spherical in shape in solution, but form elongated particles as they denature in the surface film. After this process has been completed, they may show flow-anomaly also in the bulk phase. An example is serum euglobulin. The third group consists of those proteins which show flow-anomaly neither in the bulk phase nor in the surface film. They are probably close to spherical in shape. Examples are insulin and methaemoglobin. In their third paper (17) the authors emphasize the theoretical significance of protein fiber molecules in relation to experimental morphology and cytology.

Elasticity. The importance of elasticity in protoplasmic studies has long been emphasized by Scarth (61) and Seifriz (65). In Scarth's words, "elasticity is the chief quality of protoplasm on which a conjecture of the ultra-microscopic structure of living matter can be based".

In his studies on protoplasmic elasticity, Norris (49) made a determination of Young's modulus for a strand of myxomycete plasmodium. The amount of bending of a calibrated needle with which the protoplasm is being stretched is a measure of the force exerted. He found the modulus of elasticity for strands of *Physarum* to be 9.0×10^4 dynes per cm.^{2} at room temperature for increases in length up to 40%. When the temperature was lowered to 10° C., there was an increase in the average value of Young's modulus to over twice that found at room temperature; the strands

no longer followed Hooke's law. With increases in temperature
the value of Young's modulus was lowered.

A very important observation by Pease (57), one answering a
long controversy, was that bearing on the place where the material
responsible for the elasticity of protoplasm is situated; it is not con-
centrated at the surface, as shown by tests with saponin. Pease
noted also that chloretone anesthesia has no effect on the elasticity
of *Physarum* strands, although streaming is reversibly stopped.

Contractility. Contractility is closely related to elasticity; to it
Seifriz (66) attaches much importance, particularly rhythmic con-
tractility (68) which he regards as a potential property of all proto-
plasm, manifesting itself only in tissue where it is needed, as in heart
muscle, intestines, etc. Contractility is to be seen in many forms
of cellular activity, in the migration of fibroblasts, in protozoan
flagellae, and in myxomycetes. Seifriz (67) has based a theory of
protoplasmic streaming on the rhythmic contractility of protoplasm,
on the continuous rhythmic pulsation of the protoplasm of slime
molds. He (72) states that if time-lapse moving pictures of the
streaming protoplasm of the myxomycete, *Physarum polycephalum,*
are made, a remarkable pulsation of the plasmodium is revealed.
Pictures taken every five seconds and shown at the usual rate of
sixteen per second, which means a speeding up of eighty times, show
the plasmodium undergoing rhythmic contractility and relaxation
in perfect synchronism with the protoplasmic flow. The rhythmic
contractions and expansions when thus optically accelerated re-
semble the pulsations of a heart. At each contraction and relaxation
the direction of protoplasmic flow reverses, and with the outward
flow the plasmodium advances slightly.

The mechanism of protoplasmic movement in slime molds is,
then, one of rhythmic contraction and relaxation of the plasmodium,
with a total of 95 seconds for each pulsation, 45 seconds for systole
and 50 seconds for diastole. The additional five seconds in time
of outward flow account for advancement. Kamiya (27) gives 93.7
seconds for one rhythmic period.

Seifriz (72) emphasizes the widespread occurrence of rhythmic
contractility in animate nature, in muscle, nervous impulses, indi-
vidual cells, *etc.*

Spirality. The spiral characteristics of living matter have been
but little studied, yet some observations and general conclusions
have been made.

The reviewer has long laid emphasis on the spiral qualities of living matter, whether a chromosome or tree trunk. It appears that much in nature is asymmetrical, from the carbon atom and crystals to organisms. Growth and movement take a spiral course.

Castle (14), by automatic photographic recording, studied the elongational and rotational, that is to say, spiral components, of the growth of the sporangiosphore of *Phycomyces*.

That protoplasm should exhibit spiral tendencies is obvious from the asymmetry of organisms and the asymmetry of the crystalline matter of which protoplasm is made. The extent to which spiral movement as in protozoa, spiral growth as in horns, tusks and trees, and spiral shapes as in chromosomes, can be attributed to the asymmetry of crystals, or the asymmetry of the carbon atom, or to helical organic molecules, is a matter of conjecture, but spirality in living matter is present, nevertheless, and in many forms.

Torsion. If spirality is a basic quality of protoplasm, then a state of torsion may often if not usually exist in protoplasm. Having reached this likely conclusion, research was set underway to prove it, and with surprising results. As the work done has not yet appeared in print, only a general statement can here be made. During the flow of protoplasm through a single freely suspended strand of the myxomycete, *Physarum*, the strand is twisted first to the right and then to the left as the flow of protoplasm moves first in one and then in the other direction. At each point of maximum twist a state of torsion is set up which must be relieved; consequently, there is a winding and unwinding of the strand. At the moment of each reversal in the winding and the unwinding, the state of torsion is at a maximum. The twisting of the strand is correlated with the flow of the protoplasm. The precise nature of this correlation is a particularly interesting but troublesome question which it is hoped may be answered.

Ductility. Ductility, or the capacity to be drawn out, is characteristic of protoplasm. Ordinarily it is accompanied in protoplasm by a return movement, that is to say, protoplasm is elastic, but ductility as such does not involve elasticity.

Protoplasm is highly ductile (65). If the protoplasm extended is tough, resilient and elastic, it will return with great suddenness when severed from the needle, by means of which it is being stretched. That it clings to the needle, though subjected to a sub-

stantial tension, is evidence of its high tackiness. That it often returns after being stretched is evidence of its elastic qualities, and that it may be drawn to very great lengths is proof of high ductility. The last property is great, perhaps greatest, when the protoplasm is poorly elastic, as Pfeiffer has shown (58, 59).

Tackiness. Whatever controversy may exist on the presence or absence of certain physical properties of living matter, no one has seriously questioned the existence of glutinous or tacky qualities. Protoplasm is adhesive, cells cling together. Did they not, we should, says Lewis (34), fall apart. Now the admission of tackiness carries with it admission of elasticity. These properties, and some others such as surface tension, are often confused with viscosity, it being assumed that high viscosity means high adhesive qualities, high surface tension, *etc.,* but this is not true. There is no correlation between viscosity, on the one hand, and tackiness, surface tension, tensile strength and elasticity, on the other.

The foregoing properties all owe their origin to specific structural features, tactility like elasticity being a particularly strong indication of continuity in structure.

Thixotrophy. The last manuscript written by Professor Freundlich (20) was on thixotrophy. He introduced the subject with these words: "In older textbooks of physiology, let us say of about 1900, the state of aggregation of protoplasm is left very unclear. It is frequently considered to be fluid. On the other hand, it is emphasized that life processes seem to require a well-defined 'organization' and that such an organization can hardly be imagined without some kind of a more or less solid structure. There is practically no indication of the fact that the mechanical properties of protoplasm differ essentially from those of normal liquids or solids and that they may be correlated with its colloidal nature. References to colloids are found, if at all, when the impermeability of membranes to proteins, or swelling, or the scarcity of distinct crystals, are discussed.

"This outlook has completely changed during the last twenty years. It has been found that there may exist a number of intermediate stages between the normal liquid state and the state of a crystalline solid. These are frequently observed in colloidal systems, particularly if they contain a sufficiently high amount of disperse phase. Consequently, concentrated colloidal solutions may differ essentially in their mechanical properties (viscosity, elasticity,

etc.) from normal so-called Newtonian liquids. These differences may be manifold. Two limiting cases are of outstanding importance; the first is that of thixotrophy. . . . The other limiting case is that of dilatancy. . . . Although observed and named by Osborne Reynolds in 1885, dilatancy has only recently been recognized as a remarkable counterpart to thixotrophy. Osborne Reynolds used the term when describing the behavior of moist quartz sand; it whitens and appears to be dry when the foot falls on it and becomes wet again when the foot is raised. An aqueous suspension of finely ground powder (particle size 1 to 5 μ) at a concentration of about 44 per cent of the solid is strongly dilatant. A glass rod can easily be moved through the mass at low speed, but an enormous resistance is set up if the speed is increased above a certain limit. . . . Suspensions of intact starch grains in water are strongly dilatant too. . . . The particles of a dilatant suspension are quite independent of each other; there is not the least indication of coagulation. On the other hand, the particles of a thixotrophic suspension are always found to be coagulated to a certain degree, sticking together and forming clusters. If brought into suspension in the liquid, they may be temporarily separated from each other, but they always unite again to clusters".

The biological applications of thixotrophy are many (20, 66), one of which will be discussed in the last part of this review.

The capacity of a colloidal substance to collapse from mechanical disturbance and reset instantly is dependent on specific structural characteristics. In short, a study of all physical properties of jellies, living and non-living, ultimately leads to structure.

STRUCTURE

All problems having to do with the physical properties of protoplasm lead to structure, for structure determines these properties. Protoplasmic structure is of four categories: gross structure or cellular organization, microscopic structure, ultramicroscopic or colloidal structure, and molecular structure.

Gross Structure. The gross structure of protoplasm is here taken to mean cellular organization. Scarth (62) has handled the coarser structure of protoplasm unusually well, and clarified the confusion which has arisen in the application of term after term to the successive layers of protoplasm. He distinguishes five layers or ori-

ented substances, which, though they apply to the plant cell, may fit the animal cell as well, with the exception of the outermost wall of cellulose in plants. Within the wall there are, in order, the protoplasmic membrane or ectoplast, the plasma gel or cortical endoplasm, the plasmasol or liquid endoplasm, and the inner tonoplast or vacuolar membrane surrounding the vacuolar fluid, through which kinoplasmic strands thread their way, joining the outer membrane to the tonoplast.

The presence of and need for cellular organization has been appreciated by many workers. Cohn (16) arranges the component parts of cells into patterns. Non-polarized organisms have no fixed axis of development. Polarization may be induced in initially unpolarized forms, *e.g.*, Myxomycetes and Aerasieae, by an asymmetric environment. Gause (21) considers the possible application of organization concepts of topology to asymmetric organization of protoplasm.

Skotnicky (75) states that the conducting layer of nerves is a property of the functional organization of protoplasm.

An example of gross structure or cellular organization is given by Seifriz (72) in the slime molds. In the original moving pictures of slime molds taken by Seifriz, three distinct rhythms of contraction were observed. Subsequent work by Kamiya (27) led to the postulation of many centers of contractility, that is to say, slime mold protoplasm is a polyrhythmic system. There are, therefore, many independent centers of activity, each with an individuality all its own. The physiological state of any one center differs from that of adjoining centers at any moment very much as adjoining cells in tissue differ, with this exception, that in a plasmodium the difference is less permanent.

Further indication that a slime mold is an assemblage of individual units is to be had in the habit plasmodia have of breaking up into microscopic bits at the time of sclerotium formation and during the plasmodial stage. At sclerotium formation, the drying plasmodium fragments into many small sections, the whole resembling an assemblage of cells. These subdivisions represent previous centers of activity which were physiologically distinct at the time the sclerotium was formed. It is possible that they are uninucleate masses of protoplasm. The bits which are distinct morphological units in a sclerotium cease to be so in a plasmodium, where, how-

ever, they retain their physiological individuality, one feature of which is the characteristic rhythm each possesses, a rhythm not necessarily in perfect synchronism with that of other units.

Microscopic Structure. The microscopically visible structure of protoplasm has been reviewed many times, the theories are now classical. Little new has been added, chiefly because it is now generally recognized that the visible structure of protoplasm has nothing to do with the basic structure. Thus, the visible granules and globules, which are of many categories with many names, such as vacuoles, vesicles, mitochondria, macrosomes, microsomes, *etc.*, all play important rôles in the life of the cell, but none is concerned in the structural features of protoplasm, none is a building unit, all are but inclusions. Seifriz (66) has pointed out that although the visible emulsion in protoplasm is of importance from the point of view of nutrition, it is in no way responsible for such properties of protoplasm as permeability, imbibition, elasticity or electric conduction, nor can it in any way represent that structure of living matter which distinguishes it from the non-living. The visible structure is not, as has been said in the past, a coarser manifestation of the finer structure which lies hidden from view. A denial of this does not imply that the visible structure should be ignored, for it plays a significant rôle in the metabolism of life, just as does the still coarser organization of the cell as a whole.

Ultramicroscopic Structure. No hypothesis of protoplasmic structure has been so troublesome as that designating living matter an "emulsoid". Just what an emulsoid is no one seems to know. Gelatine is called an emulsoid, but the word means "like an emulsion", and gelatine is not like an emulsion. The emulsoid hypothesis of gel structure long prevailed in biology because of support from some chemists, among whom was Gortner (23), whose book, excellent in parts, was accepted in toto by some few chemists and biologists. Gortner, in his classification of colloidal systems, was not justified in grouping the glue-like systems, and organic colloidal jellies in general, with colloidal suspensions of metals, thus grouping colloidal gold with glue, egg-white and agar. These two types of systems have little in common. Duclaux (18), in the introduction to his book, "Les Colloïdes", states that he will not consider the metallic suspensions, as they have nothing to do with true colloids. Thomas Graham, in naming colloidal systems, had the glue-like sub-

stances in mind, for that is what colloid means. So drastic a point of view as that of Duclaux can not be taken, for it is impossible to exclude the lyophobic colloidal suspensions from the colloidal world, if only because of their historical background. Suspensions have little in common with glue-like colloids. To place the latter in the same class with the former is to fail to understand the true characters of both groups. Attempts to apply the term "emulsoid" have been the cause of much confusion. Seifriz (68) has brought this bewilderment to light and suggested, as have many chemists, elimination of the term. The concept is already dead, except in some few minds.

With visible structural features obviously too coarse to satisfy a stereochemical point of view, there remains only the molecular line of attack, that pursued by the chemists.

Biologists in their interpretation of protoplasmic structure have done no more than apply to living matter accepted theories on the structure of organic matter, of proteins, cellulose, rubber and wool. There has been some strong antagonism to all attempts to correlate the known structure of organic non-living matter and the possible structure of living matter. This opposition is apparently based on prejudice against any interpretation of the basic molecular structure of protoplasm. Investigations and hypotheses on protoplasmic structure are termed futile, just as were the experiments of Pasteur and Langley, and much other pioneer work in science. The opinion that studies on and an interpretation of the molecular structure of protoplasm are futile is held by certain chemists who are having difficulty with the structure of proteins, enzymes and pigments. It is their belief that any attempt to interpret protoplasmic structure must wait until work on non-living systems is completed. To this advice there are three replies: their work will never be done and protoplasmologists are not willing to wait that long; no branch of science has ever waited for another to advance; it has often been the less well grounded sciences which have shown the more exact sciences the road to follow, as in the cases of osmosis, ionization, solution laws, pH, hormones, vitamins and viruses. In each of these subjects the biologist set the problem and went quite a way with it, and at that point the chemist took it up. In the same way, the biologist will continue his studies on the molecular structure of protoplasm, grateful for the help of many able physicists and chemists and not much minding the destructive comments of a few.

Certain types of research have contributed much to our knowledge of protoplasmic structure, and it will be well to consider them under their respective headings.

X-Ray Analysis. Of late, X-ray analysis has contributed more to the study of protoplasmic structure than any other method of physical-chemical research; and it may also be said that one man has been primarily responsible for this research. Astbury (2, 3) has maintained a deep and constant interest in the application of his work to biology and has coordinated much of the research of physical-chemists and biologists.

The X-ray work of Astbury which has biological applications was originally done on wool, and later on a number of kinds of organic material, in particular, the muscle protein myosin. To properly understand the theories of Astbury and of others (4, 5, 37, 41) who have done similar work, it is necessary to recall both the familiar polypeptide chain and the newer concept of the cellulose fiber. Although the protein molecule is the structural unit of protoplasm, it is the cellulose fiber which first made biologists aware of the basic structure of living matter. The polypeptide chain is the older concept, but the imprint it left on structural chemistry was insufficient to carry us through to the stage now reached. The cellulose chemists succeeded in establishing the linear unit as the basic one in organic matter.

The botanist Sponsler (77) did much to bring about a revival of stereochemical thinking in biology. It was he (76) who first established with fair certainty the existence of the cellulose molecular fiber. Out of his work and that of others simultaneously carried on in Europe (37, 38, 41, 42, 78), there arose such concepts as the molecular brush heap, then a more orderly parallel alignment of fibers, and finally the overlapping of cellulose molecular threads. The last of these concepts best explains the high tensile strength of some natural fibers. The at one time popular micellar hypothesis of Naegeli has been greatly modified, and by some discarded. There is, however, considerable optical evidence based on observations made with nicol prisms, that a micellar structure is present in natural cellulose. Furthermore, the crystallites described by Astbury and others in muscle are a substantiation of the micellar hypothesis in protein.

Not only did hypotheses of the molecular structure of cellulose

lead ultimately to the concept of a fibrous structure of living matter, but microscopic observations as well made their contributions. These observations revealed minute fibrillae in natural wood. Evidence of fibrillae in natural cellulose is of various sorts. By microdissection Seifriz and Hock (73) demonstrated the presence of minute fibrils in paper pulp.

The protein fiber concept is not new, but shape and orientation of the fiber had yet to be established. This the X-ray chemists accomplished. It has long been customary to draw the diagram of a polypeptide chain in a broken zig-zag line, probably in the belief that the molecular thread is not fully extended, and this is what Astbury found to be true. But the folding is not always pronounced. The extent of folding is determined by environmental conditions. Astbury (3, 4) states that keratin, as it exists in unstretched mammalian hair, is in what he calls the a state. On stretching, the hair molecular backbones become completely extended and the keratin is then in the β state. If relaxed hair is treated with steam, the keratin threads become supercontracted. These states may not be produced by such simple foldings of the backbone as Astbury originally postulated, but the state of affairs is probably very much of this sort. Astbury (3) has presented evidence to show that myosin in relaxed muscle is in the a state, and that on muscular contraction it becomes supercontracted.

Before turning to the orientation of protein fibers in mass, which involves the important question of the lateral bond, it will be well to add evidence of another kind in support of the general concept of the molecular fiber. Included in this evidence is the excellent work of Bensley (7–10) and his collaborators (25). Their research is a chemical attack on protoplasmic structure, as distinct from the physical one of X-ray analysis. Bensley (7) sought to obtain from the cell what others had avoided because of the difficulties of solubility. Using frozen dried material Bensley and Hoerr (11) were able to introduce solvent solutions into a chemically unaltered cell. In such preparations of the liver of the guinea pig sodium chloride quickly dissolved the mobile protein of the cell but left behind a morphologically complete cell. Treatment with ammonia solution removed the mitochondria and nuclear chromatin but left the cell membrane, cytoplasmic substrate, nuclear membrane and linin threads. The cytoplasmic residue was termed "structural protein".

On repeated solution and precipitation with strong alkali a material named "ellipsin" was obtained. Later Bensley (8) reported the discovery of a sodium chloride soluble component of ellipsin, a highly viscous substance with pronounced elastic properties containing discrete fibers of great length. This he called "plasmosin", regarding it as the structural foundation of tissue very much as in myosin in muscle.

Banga and Szent-Györgyi (6) obtained a similar viscous extract from kidney which precipitated in fibrous form and gave an X-ray diagram like that of myosin β.

Purification of plasmosin by Lazarow (31) led to the discovery that plasmosin is a nucleoprotein, a fact which would suggest its origin from the nucleus. The idea that nucleoproteins are confined to the nucleus has, however, been breaking down, owing to the discovery that many active components of cells have a nucleotide structure and the discovery of Claude (15) that the particulates contain nucleoprotein. Without rejecting the idea that the nuclei contain plasmosin, Bensley (10) believes that it is, in large measure, a constituent of cytoplasm. Whether it originates there or is produced in the nucleus is, of course, a topic for speculation and research.

In contradiction to the foregoing conclusion as to the seat of origin of plasmosin, Mirsky and Pollister (44) insist that the preponderance of evidence indicates that the great bulk of fibrous nucleoprotein comes from the nucleus, that the cytoplasm can at most contain only traces of it. Furthermore, it is also maintained that this fibrous precipitate extracted by Bensley from cells only superficially resembles the well known fibrous cytoplasmic protein myosin, but conforms very closely to substances known to come from cell nuclei.

The work of Bensley and his collaborators leads to the same conclusion as does the work of the X-ray chemists, namely, a fibrous structure of the basic constituents of protoplasm. Following Bensley's suggestion (9) to modify Meyer's (42) summary of Seifriz's (69) views, we arrive at the generally accepted conclusion that certain of the structural units of protoplasm are linear molecules, or possibly aggregates of molecules, so arranged as to form a network of primary valence chains tied together at numerous points by chemical bridges held by molecular cohesion, residual valences or hydrogen bonds.

Into the biological-chemical interpretation of protein behavior has been introduced the term "denaturation". As denaturation is little understood, a general characterization of it may be given to advantage, and this is well done by Lawrence *et al.* (29) in the following words: "The conception of protein denaturation is essentially the transformation of compact, rigid almost crystalline, coiled or folded polypeptide-chain molecules with a highly specific configuration, maintained by secondary valences, hydrogen bonds, sulphydryl linkages, etc., into unrolled, more flexible, fibrillar molecules with a less specific configuration. . . . The decrease in solubility on denaturation is explained by the assumption that in the native molecule the polar groups are mostly on the external surface of the framework, but when the molecule unrolls, the non-polar hydrophobe groups are bared. The appearance of $-SH$ groups is explained by their being no longer employed in cross-chain linkages. The crystallization of native proteins and the incapacity of denatured proteins to crystallize is explained by the view that they are no longer rigid and compact structures. On the other hand, the denatured fibrils may participate in forming regular submicroscopic bundles, or liquid crystals. . . . Drying withdraws water molecules from the framework causing it under some conditions to crumble; heat and radiant energy shatter the secondary valency bonds; shaking and gas bubbling uncoil the frameworks at the air-water interfaces; urea and guanidine derivative molecules penetrate into the frameworks and disrupt them; organic solvent molecules also penetrate and by dissolving the non-polar groups, turn the frameworks inside out".

We have, therefore, to do with two forms of the protein molecular chain, an extended and a folded form, and possibly a closed ring or globule.

We may now turn to the orientation of these chain molecules in mass. There are many possibilities: a haphazard brush heap of short fibers, an entanglement of long threads, a symmetrical orientation resembling bricks in a wall, a parallel alignment of long and overlapping rods, or a grid structure. All have been postulated, and each meets certain conditions (67). Astbury (4) advocated the grid, by which is meant that the fibers are in parallel alignment, and the plates thus formed are stacked one upon the other. In changing from the extended to the folded form, the entire grid folds

with the individual fibers. This is the basis of modern theories of muscular contraction to which reference has been made and of which more will be said later.

Into this rather well substantiated view of protoplasmic structure, there have crept some novel features, certain ones of which are enlightening, others disturbing, though not seriously so. Among the latter is the concept of the spherical molecule, so ardently supported by Wrinch (82). To build a structure possessing elasticity and related qualities out of spherical units is impossible. Comparison may again be made to a sand pile, on the one hand, and to a brush heap, on the other hand; the latter, not the former, is elastic. Meyer and Hohenemser (43) have shown that certain protein molecules, for which a spherical form has been claimed, cannot be so formed. Lecomte du Noüy (32) likewise believes that oriented albumin, the existence of which has been demonstrated by him, Devaux and Langmuir, contradicts the spherical shape. Lecomte du Noüy regards the albumin molecule as short and prismatic in shape. Banga and Szent-Györgyi (6) tentatively settled the controversy by assuming that "whenever nature needs a mobile protein (serum albumin and globulin, secretions like milk-proteins, hormones like insulin, different enzymes, etc.) it applies the globular shape, and whenever it wants to build a solid structure it applies the rod shape. Proteins have been found to be mostly globular because unconsciously the mobile and more easily accessible proteins have been selected for study".

To the convincing hypothesis of Banga and Szent-Györgyi may be added the further possibility of the conversion of the globular form into the linear form. Lawrence, Needham et al. (29) support this concept in saying: "If denaturation be taken as synonymous with the unrolling of a corpuscular protein, this must occur at all oil-water interfaces in the cell, e.g., at the surfaces of mitochondria, the cell membrane, the intracellular oil droplets, etc.". To this suggestion they add: "If, then, we may adopt the hypothesis that protein molecules unrolled into elongated fibrils by denaturation have a part to play in the dynamic architecture of the cell, as well as those which are elongated before denaturation, the question arises as to how they are produced in the living organism? Is it possible that the rotational churning and streaming motion of protoplasm has some connection?".

The Lateral Bond. One of the most interesting and important features of the specific type of structure here postulated, a structure the chief characteristic of which is its continuity, is the lateral bond. Seifriz (71) lays emphasis on the hydrogen bond as the most probable of lateral ties in living and non-living organic matter. He says: "The problem of the simultaneous presence in organic systems of fluidity and structural continuity is one which confronts the chemist as much as the biologist. Highly branched, cross-linked, three-dimensional polymers always exhibit elasticity, though fluid. Side chains which make lateral connections between molecules have long been recognized in stereochemistry. But the concept that one end of the link is loosely attached and the other firmly so is new and of great biological importance. It has been suggested that variations in the degree of hydration of the side chains constitute a possible explanation of a weak attachment at one end. But it is another interpretation of this feeble union which I have selected, namely, the hydrogen bond".

In a chemical cycle, as in muscle, during which accessory molecules go in and out of combination with the side chains of the protein main chains, the latter take up a cycle of configurations. In all such dynamic systems, of which protoplasm is one, there is a constant shifting of ties between the structural units. The loose union which permits this is very probably a hydrogen bond. Linus Pauling (56) believes this bond to be of greater significance for physiology than any other single structural feature. It serves our present purpose well because it is not a strong bond.

When an atom of hydrogen is attached to not one but two other atoms and thus acts as a tie between them, the union involves the hydrogen bond. In such a case, one of the points of attachment is a firm covalent bond, and the other a weaker one, essentially ionic in character. The latter is the hydrogen bond.

In general, the hydrogen bond is part of a situation in which hydrogen appears to have two valences, one stronger with some ionic character, the other weaker with more ionic character. The strength of the latter weaker bond ranges from one-tenth to one-hundredth that of the former, according to the atoms involved and their distance apart. Because it is feebly attached, the hydrogen bond shifts easily from one atom to another.

The use to which the hydrogen bond may be put in interpreting

protoplasmic behavior in respect to structural continuity is illustrated in a simple system like water. For some time it has been known that the water molecule is polar, *i.e.,* it has an electric moment. It possesses, therefore, the characteristics necessary for molecular orientation. The two hydrogen atoms are positively charged and the oxygen atom is negatively charged. At a distance, such a molecule is electrically neutral, but viewed nearby, it is positive or negative depending on one's position. The negative oxygen atom of a water molecule will attract and hold the two positive hydrogen atoms of another water molecule and with a force which may equal the hold on its own two hydrogen atoms. The valence bonds within a water molecule may be regarded as chemical and those between adjoining molecules as physical, but it amounts to essentially the same thing in the end, for the hydrogen atoms and the oxygen atoms exert forces on the surrounding molecules which are no less chemical in nature than those holding the atoms within the molecule together. Fluidity depends upon the shift that takes place between one molecule and another. Water molecules are always in partnership, but always changing partners. We thus see how polarity leads to orientation and it in turn to continuity in structure in liquid systems. That this is true is indicated by the different patterns which certain solutions yield, indicative of some kind of arrangement.

In a similar manner, Pauling interprets the structural continuity of water in terms of the hydrogen bond. Instead of the classical H_2O, it is presumed that each oxygen in water is surrounded by four hydrogens, two of which, close to the oxygen atoms, are joined to it by primary valences, and two, farther away, by the hydrogen bond.

The situation existing in water is even better applied to proteins where structural possibilities are infinitely greater; and proteins are the building material of protoplasm. In amino acids the nitrogen atom has one open coordinate position with which it may unite to a hydrogen in the same or another molecule. This union is a hydrogen bond; it may join the nitrogens of two amino acids.

The essential feature of the hydrogen bond, insofar as our present problem is solved, is its ease in shifting. This permits fluidity though maintaining continuity in structure. But protoplasm does not flow with equal ease at all times; it is often of high viscosity,

and it may be firm. The lateral bonds are, therefore, more securely held at one time than at another, and at still other times they are tighly locked, preventing flow. If the hydrogen bond satisfies these conditions, it must show considerable variability in firmness, from a feeble contact permitting ready readjustment, to a firm grip tightly locking the fibrous units of the living three-dimensional lattice. Pauling (56) points out that firmness of the hydrogen bond varies with the electronegativity of the atom.

As one of the essential points of this discussion is the variability in firmness of the weaker end of cross-chains, we may dwell upon it just a moment longer in order to emphasize an important difference between this variability in non-living and in living systems. Usually, variability in the viscosity, elasticity and tensile strength of a solution of non-living chain polymers is accomplished simply by altering the concentration. At high concentration the chains join at more points. But in protoplasm the "concentration" remains the same, yet the physical properties vary. This is accomplished by a change not in concentration but in the firmness of the cross ties. How this change may come about has been suggested. There is the further possibility that protons are added or subtracted with change in pH, forming or breaking hydrogen bonds; change in salt concentration might also alter their strengths.

The hydrogen bond provides a mechanism by means of which continuity in structure, elasticity and rapid changes in viscosity in protoplasm may be interpreted.

Long molecular chains and suitable lateral bonds make possible one of the most characteristic properties of protoplasm, its capacity to exist as a gel. A striking comparison between protoplasm and many of the more extended proteins is the capacity of both to form gels at low concentrations. Staudinger (78) has emphasized that it is not enough for a particle to be rod-shaped; to form a gel it must be able to form cross-linkages and a net structure. Chains of polystyrene are dispersed by organic solvents. Staudinger found that if as little as 0.002% of divinyl-benzene is added to the styrene before polymerization, cross-linkages are produced. The resultant polymer, although indistinguishable by chemical tests from pure polystyrene, when placed in the same solvents swells enormously but does not dissolve. In the case of a protein, like gelatin, gel formation depends on cross-linkages between the main chains. These bonds

may vary in strength, from primary valence bonds, such as salt and disulfide linkages, to van der Waals' forces.

The need of a continuous and open network in protoplasm is shown by many of its properties, among which is its high water content. Over 90% of some forms of living matter is water. If so much water is to be held by so little solid matter, the sponge-like framework must be very open and very secure.

The physical properties of living matter have been briefly reviewed by Norris (51), and somewhat more extensively by Taylor (80).

BIOLOGICAL APPLICATIONS

Anesthesia. Seifriz (70) has reinterpreted and given experimental support to the theory of anesthesia advanced by Claude Bernard. Bernard had said that anesthesia results because of the coagulation of protoplasm, but as coagulation is usually irreversible and therefore incompatible with life, the theory was discarded. The difficulty lay primarily in the use of the word coagulation. Had Bernard said gelatinization or a pronounced increase in viscosity, the theory would have attained greater standing. The lipoid solubility hypothesis replaced that of Bernard. The theory of Seifriz rests solely on experimental observations. The mechanism of anesthesia, as set forth by Seifriz (72), is one of thixotrophic setting or rapid gelatinization of the protoplasm followed by natural solation on recovery. Seifriz thus broadened the usual concept of thixotrophy by laying added emphasis on one of its features. Thixotrophy, as originally described by Schalek and Szegvary, referred to the sudden collapse of a gel due to mechanical agitation. As instantaneous resetting of the sol is equally characteristic of thixotrophic substances, thixotrophy may be defined as the instantaneous collapse and rapid regelatinization of a colloidal substance. Rapid solation and gelation both occur in protoplasm.

If the protoplasm of a myxomycete is treated with a favorable anesthetic agent, *i.e.*, one causing no observable damage and permitting recovery, such as carbon dioxide, cyclopropane and chloroform, the protoplasm ceases flow very rapidly. There is little warning, occasionally a momentary tremor, and then a sudden and complete stopping of flow. On removal of the anesthetic agent there is resumption of normal activity with no observable damage (70). The instantaneous cessation of protoplasmic streaming caused by

carbon dioxide appeared to involve a setting or gelatinization of the protoplasm. It also seemed likely that the failure of other anesthetic agents to put a sudden stop to protoplasmic streaming was an indication that no gelation had taken place, but there was no direct proof of either deduction.

By means of an ingenious technique involving the application of pressure, Kamiya (26) was able to oppose and control protoplasmic flow. If protoplasm is quiet, due to anesthesia, pressure externally applied should cause it to move if it is in a liquid state, and fail to move it if it is firm. External pressure failed to move protoplasm anesthetized with carbon dioxide.

From this we may conclude that anesthesia is due to the thixotrophic setting of protoplasm. In the case of higher vertebrates only nerves would be affected, whereas in slime molds all the protoplasm sets thixotrophically when anesthetized.

The chief virtue of a purely physical interpretation of anesthesia lies in the fact that it fits all forms of anesthesia by whatever means produced. This no chemical hypothesis can do. For example, lipoid solubility well explains the anesthetic and destructive effects of fat solvents on protoplasm, but it can not meet a situation in which a state of anesthesia is brought on by a salt, by carbonic acid or by shock. An hypothesis which states that the protoplasm of nervous tissue in higher organisms becomes firm or of very high consistency when in a state of anesthesia, no matter how it is produced, will fit all cases of anesthesia whether brought on by ether, magnesium chloride, carbon dioxide, cold or shock.

That a state of anesthesia should exist when protoplasm has been gelatinized is evident from the fact that with an increase in viscosity there must be a decrease in metabolic activity which reaches a minimum at maximum viscosity or reversible gelatinization of the protoplasm. Lhérisson (35) has pointed out that metabolic rate is inversely proportional to viscosity. He based this conclusion on the fact that the rate of chemical reactions is dependent upon diffusion and the rate of diffusion is proportional to viscosity.

An interesting field of speculation is that involving phenomena which border on surgical anesthesia as ordinarily practiced. Unconsciousness when produced by shock, cold, fear, indeed by any means, is in each case a form of anesthesia; the extremes would then be natural sleep and the coma that precedes death. The last may be

brought on by so simple a process as a sharp drop in blood acidity. Among these exceptional forms of anesthesia that by cold is recognized by the physician. Seifriz and Epstein (74) studied shock anesthesia by allowing drops of water to fall from different heights striking slime mold protoplasm. From the values for gravity, the drop mass, the height from which the drop fell, provided it did by its impact stop protoplasmic flow with subsequent recovery, were computed the force necessary to cause anesthesia by mechanical shock. Electrical shock produced similar results.

In all forms of gelation or gelatinization, and subsequent solation of protoplasm, a locking of lateral bonds, holding fibrous structural units more or less firmly together, is involved.

Genetics. The possible genetical applications of theories on protoplasmic structure are great; at least, this would seem to be inevitably true in the face of modern views on a permanent linear arrangement of genes. The ideas of continuity in protoplasmic structure, advanced by Seifriz (66), are in keeping with genetical concepts of a fixed arrangement of genes. There is less need of a mobile structure in chromosomes than in the case of cytoplasm.

As chromosomes are a form of protoplasm, they must resemble it in a general way, that is to say, the basic physical qualities of the two must be essentially the same. This is true with but minor modifications. Thus the substance of which chromosomes consists is more viscous than the surrounding medium; the viscosity apparently remains high. Chromosome material is likewise elastic and glutinous.

Buck (13) has subjected the chromosomes of the salivary gland of *Chironomus* to microdissection. He states that they show considerable rigidity, withstand a several-fold elongation without breaking, and decrease in diameter when stretched.

The visible structure of protoplasm is often alveolar (66). Metz (40) has shown that this is true of chromosomes. The giant salivary gland chromosomes of Diptera are alveolar in structure.

Permeability. The selective permeability of cells is generally handled as a subject apart from structure, but several of the classical theories of cellular permeability involve mechanisms, and mechanisms involve structure. Thus the sieve hypothesis of selective permeability is a purely mechanical interpretation; indeed chemical hypotheses such as those involving the solution of fats are, in the

last analysis, structural interpretations. The making of models
which emulate the behavior of the cell membrane is likewise a prob-
lem in the physical structure of protoplasm.

It is not the reviewer's intention to summarize the subject of
cellular permeability, but merely refer to several articles which help
toward a better understanding of the structure of the outer surface
of the cell. One of these publications is that of Lundegardh (36).
He denies that materials enter cells only as molecules. In view of
the modern concept of the complete ionization of salts, even at rather
high concentrations which are never reached in the soil, it is
doubted that there are any molecules present of the strong electro-
lytes needed by the plant; therefore salts must enter as ions. To
cite that carbon dioxide is a weak electrolyte and but partially dis-
sociated, has no bearing on the problem as a whole, for carbon
dioxide is but one of many substances which enter cells. Lunde-
gardh says that if a cell is placed in contact with the solution of a
single salt, full equivalency in the absorption of cations and anions
is an exception. "These experiments show that the salt is not taken
up as molecules . . . ".

Lundegardh (36) states that the apparent contradiction of a lipoid
surface film on cells and the entrance of water and ions through it,
is best explained in terms of a monomolecular layer of fatty acids
such as those studied by Langmuir. Rideal (60) states that such
a monolayer will allow water molecules to pass through it, yet the
electrical resistance must be high. In short, the conflicting experi-
mental data are to be explained only in terms of structural char-
acterics of the cell·surface.

Protoplasmic Streaming. An interpretation of the mechanism
responsible for protoplasmic flow must certainly rest on structural
features: thus, the now classical solution-gelation hypothesis of
amoeboid movement, advanced by Mast (39), is based on gross
structural features such as those outlined by Scarth (62) and re-
viewed in this paper. The rhythmic contractility hypothesis of
protoplasmic streaming, advanced by Seifriz (67), is based on
molecular structural features involving the contraction of folded
protein chains.

Pease (57), as a result of his studies on the effects of hydrostatic
pressure, suggests that protoplasmic streaming is due to local rhyth-
mical contractions of the plasmogel.

The subject of protoplasmic streaming has been recently reviewed in this journal (Vol. 9: 49. 1943).

Frost Resistance. Scarth (63) and his co-workers (33) have given us a protoplasmic interpretation of frost resistance. They state that the key factors in resistance are protoplasmic. Other factors reinforce the protoplasmic one, but by themselves are ineffective. Most important of all is the reduced tendency of the ectoplasm to coagulate. This property protects against injury; thus it determines the minimum temperature the plant can withstand. There is no evidence that coagulation is prevented to any degree by an increase in substances such as sugars, or a decrease in pH or electrolyte concentration. The change would seem to be in the labile colloids themselves. Increased toleration of plasmolysis and increased survival after rapid deplasmolysis following non-lethal plasmolysis, invariably accompanies hardening against cold. Resistance to plasmolysis and deplasmolysis is greatest in very hardy cells and is roughly parallel to frost and drought resistance in wheat. Osmotic pressure and hardiness frequently vary independent of each other and never show the excellent correlation of protoplasmic changes.

Seifriz (72) concludes: "It is truly an encouraging sign in the progress of science when properties of protoplasm such as contractility and structural organization, which heretofore were so little understood, can now be interpreted in terms of folded polypeptide fibers, interlocking side chains, hydrogen bonds, and asymmetry of the carbon atom". To these biological processes capable of interpretation in terms of structure, may now be added anesthesia, protoplasmic streaming and frost resistance.

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THE GENETICS OF BRYOPHYTES. II[1]

CHARLES E. ALLEN
The University of Wisconsin

A paper under this title (7) reviewed the literature in the field approximately to 1934. The present account attempts to cover, so far as the conditions of the time render it possible, that which has since appeared.

HETEROPLOIDY

Various reports indicate the occurrence of polyploid series of related species among bryophytes as among plants of other groups. There are occasional indications also of the occurrence of different chromosome numbers in strains referred to a single species. *Riccia fluitans* has been recorded as having 8 (95) and 14 or 15 chromosomes (29). Both *Calypogeia Neesiana* and *Haplozia lanceolata* are credited with 9 (95) and 16 to 18 (29). *Dumortiera hirsuta,* previously reported with 9 (29, 45) or 10 (54), is found (89, 93–95) to occur in Japan in 3 forms having, respectively, 9, 18 and 27. The triplont is more widespread than the haplont or the diplont and appears upon a wider range of geological formations. A diploid form with 18 chromosomes is reported (89) from Tennessee. *Polytrichum commune* has 7 (35); var. *uliginosum* 7 and an unnamed variety 14 (43). Races of *Bryum caespiticium* occur with 20 instead of the typical 10 chromosomes (98, 99).

Aneuploid races are apparently infrequent in nature. In *Pallavicinia Lyellii,* whose usual haploid number is 8, 9 chromosomes were found (104, 105) in gametophytes from North Carolina and Texas. Sporophytes from the latter source had 18. In 16-chromosome sporophytes from Virginia, irregular meioses were observed. Such divisions may account for the origin of spores with unusual chromosome numbers.

Two gametophytes of *Calobryum rotundifolium* had 10 chromosomes instead of the usual 9 (78, 95). A gametophyte of *Sphaerocarpos Donnellii* with a constant chromosome number of 9 instead of 8 developed from an irradiated spore (39). Individuals of this species which are euploid in terms of autosomes but aneuploid with respect to sex chromosomes will be considered later.

[1] Supplement to article in The Botanical Review 1: 269–291. 1935.

Doubling of the chromosome number may occur in either generation. Diploid branches have appeared on haploid gametophytes of *S. Donnellii* (39). A suggestion as to the possible method of such doubling is supplied by Mackay's (52) observation of a cell in this species whose chromosomes in the diploid number were arranged in pairs. The figure is similar to some in the root tips of various angiosperms which are characterized by a spontaneous doubling of the chromosome number (polysomaty). The development of some diploid and some haploid gametophytes from the spores of one sporophyte of *S. Donnellii* led Knapp (39) to conclude that a doubling of the chromosome complement had occurred locally in the sporophyte.

Giant spore tetrads appeared in some crosses involving mutants and tropical species of *Marchantia* (15). These also were explained by a local occurrence of tetraploidy in the sporophyte. Occasional spores of *Aspiromitus sampalocensis* produced diploid gametophytes (56). These were smaller than haplonts but had larger cells, and the ♂ ♂ bore larger antheridia. A gametophyte of *Calypogeia Neesiana* (95) bore 1 diploid among many haploid gemmae.

By treatment of gemmae of *Marchantia polymorpha* with colchicine, Satina (cited by Blakeslee, 13) obtained diploid ♀ and ♂ gametophytes. In gametophytes of *Pallavicinia Lyellii*, after immersion in colchicine solutions, Wolcott (105) observed diploid and tetraploid cells as well as others with the original haploid complement.

Regeneration of a phenotypically ♀ diploid gametophyte from the foot of a sporophyte occurred a few times in Burgeff's (15) experiments with *Marchantia polymorpha*. He obtained similar results readily in some tropical forms of *Marchantia*. Diploid gametophytes thus produced from hybrid sporophytes (*M. emarginata* var. *multiradia* × *M. calcarea*) were mated with haploid ♂ ♂ of both parent forms. From the resultant sporophytes gametophytes were regenerated; all were triploid except 1 tetraplont. Spores of 1 sporophyte from a mating of this type yielded 1 triploid gametophyte (with 27 chromosomes), 1 hypotriploid (with 24), 9 diploid (with 18), 2 hyperhaploid (with 10), 2 haploid (with 9). Diploid gametophytes of *Anthoceros tjipanasanus* have likewise been obtained by regeneration (56). They were comparable to those previously reported (61) in *A. laevis* and *A. Husnoti*.

Diploid protonemata regenerated from sporophytes of *Splachnum rubrum* and *S. vasculosum* bore diploid gametophores (10). Similarly regenerated diploid protonemata of *Phascum cuspidatum* (72) produced variously abnormal shoots; these bore no archegonia, but some bore antheridium-like organs. The leaves and stems of such shoots gave rise to sporophytes from whose spores arose protonemata and gametophores. Some of the latter resembled typical gametophores; others were abnormal. It was concluded that meiosis, often irregular, had occurred in the apogamously developed sporophytes.

In polyploid series derived, respectively, from *Funaria hygrometrica* and *Physcomitrium piriforme,* the ratio of total plastid area to cell volume diminishes rapidly with increase in number of genoms (60). However, in polyploid series involving genoms from both species the diminution is much less rapid.

The most frequent cause of the appearance of diploid gametophytes, at least in hepatics, is the production by sporophytes of diploid spores. In species and races of *Sphaerocarpos* whose spores regularly remain attached in tetrads, it is well known (7) that spore dyads occasionally, but in varying proportions in different strains, replace tetrads. Less frequently triads and monads occur. Other irregular spore complexes are sometimes found (37, 39). Each spore of a dyad, or the large spore of a triad, if it germinates, usually gives rise to a diploid gametophyte. X-irradiation of spore mother cells of *S. Donnellii* before meiosis (37) results in the appearance of an increased number, approximately proportional to the radiation-dosage, of aberrant complexes.

In *Pallavicinia Lyellii,* especially in some races, occasional spore dyads occur (105). Apparently the spores of such dyads do not separate in the course of development as do the spores of tetrads in this species. Immersion in colchicine solutions of gametophytes bearing young sporophytes induced irregularities, including production of dyads and monads.

Spore dyads occur in *Marchantia polymorpha, M. alpestris* and some tropical species of the genus (15). Germination of dyad spores gives rise to diploid (rarely to haploid) gametophytes. (Apparently Burgeff's statements on this point, as well as those concerning tetrad spores, are based upon their germination before full maturity and hence before their separation.)

That the production of less (or more) than 4 spores from a spore mother cell must be consequent upon some meiotic irregularities is obvious. Nothing is known as to the nature of these irregularities in *Sphaerocarpos*. Lorbeer (45, 48) thinks that dyad-formation is due to "apomeiosis", defined as "a mitosis with the omission of a numerical reduction division". He has reported (48) artificial induction of apomeiosis in *S. terrestris* (*S. Michelii*), but without mention of the methods employed. From the sexual conditions in some of the gametophytes coming from dyad spores of *S. Donnellii*, Knapp (39) concludes that "apomeiosis" can not explain their formation. Burgeff (15) thinks that the origin of dyads in *Marchantia* is due to abnormal wall-formation after meiosis.

Only in *Calobryum rotundifolium* have the divisions in spore mother cells, leading to the formation of diploid nuclei, actually been observed (78, 95). The first division is not completed; a restitution nucleus is formed, which then divides equationally. Tatuno suggests that the checking of the first division may have been due to low temperatures at the time of its occurrence (early February).

Triploid and tetraploid sporophytes of *Sphaerocarpos Donnellii* have been obtained, the former by matings of diplont × haplont (5, 6, 39, 46, 52, 53), the latter by matings between diplonts (39, 52). In matings of (supposed) diploid with haploid gametophytes of *Marchantia* species (15), triploid as well as some diploid sporophytes were obtained. Matings of diplont × diplont produced tetraploid, triploid and diploid sporophytes. The explanation suggested for the occurrence of triplonts and diplonts is a loss of one or two genoms. Some triploid sporophytes produced tetrads of diploid spores, supposedly by addition in the sporophyte of one genom.

Somewhat abnormal sporophytes resulted in *Aspiromitus sampalocensis*, presumably from a fertilization of diploid eggs by haploid antherozoids (56). One such sporophyte produced spores which yielded 175 haploid and 42 diploid gametophytes.

MONOECISM AND DIOECISM

Any bryophytic species is either dioecious or (in a broad sense) monoecious; a spore transmits to the gametophyte arising from it one sexual potentiality only or the potentialities of both sexes. It

is relatively simple to determine which of these conditions holds in a hepatic, although in an apparently dioecious species only extensive culture can demonstrate whether or not dioecism is absolute. Monoecious mosses, however, present a variety of arrangements as concerns sex organs. The term applied to these different arrangements, as to some of which usage is not entirely consistent, are avoided in the present discussion. If both archegonia and antheridia are borne, however distributed, on the same leafy shoot (the "moss plant", here designated the gametophore), the species is monoecious, although external conditions may favor the appearance of the organs of but one sex. Greater difficulty arises when some gametophores of a species bear only archegonia, others only antheridia. Such species are often described indiscriminately as dioecious. However, if a single protonema can produce both ♀ and ♂ gametophores, the species is monoecious. If one protonema can give rise only to gametophores of one sex, the species is dioecious. Only by the culture of single protonemata, either primary (from spores) or secondary (by regeneration from gametophores), until gametophores appear, can the question of the genetic separation or non-separation of sexual potentialities be settled. In this manner it has been shown (10), in confirmation of earlier results (14, 62), that *Splachnum pedunculatum* (*S. sphaericum*) is dioecious, and that so likewise are *S. melanocaulon*, *S. luteum*, *S. rubrum* and *S. vasculosum*. *S. ampullaceum* and *S. australe* are monoecious. In *Pogonatum subrotundum* (*P. nanum*) and *P. aloides*, Schratz (59) found, in addition to ♂ gametophores of varying size arising from protonemata on soil, dwarf ♂♂ growing on older gametophores (some certainly ♀). He concluded, admittedly on incomplete evidence, that the dwarf ♂♂ arose from secondary protonemata produced by the ♀ gametophores. If he is correct, these two species are monoecious.

In some dioecious hepatics, the ♀ plant is noticeably larger than the ♂. This has long been known in species of *Sphaerocarpos*; it has been shown to be true in *Aspiromitus sampalocensis* (56). In many Musci with separate ♀ and ♂ gametophores, whether the species is monoecious or dioecious, the same rule holds. In some species some or all ♂ gametophores are minute (dwarf ♂♂).

A single protonema of *Acaulon muticum* produces both ♀ and ♂ gametophores (101), the species being, therefore, monoecious. The

♂ shoots, while smaller than the ♀, are not so minute as to be designated dwarf ♂♂.

Classical examples of dwarf ♂♂ are those of *Leucobryum glaucum,* living on leaves and stems of larger gametophores of the same species. Larger ♂ gametophores, however, occur on soil. Some secondary protonemata of this species (102, 103) bore ♀ gametophores, some ♂. A single protonema never produced both. The species appears, therefore, to be dioecious. The first ♂ gametophore produced by a protonema is relatively large, though somewhat smaller than and different in habit from a fully developed ♀ gametophore. Later ♂♂ borne by the same protonema are progressively smaller; the final ones are dwarfs. In this, as in other dioecious mosses, except some species of *Macromitrium,* no constant visible differences have been detected between ♀- and ♂-producing spores or protonemata.

In *M. Blumei,* Fleischer (23) found dwarf ♂♂ in abundance on leaves and other parts of ♀ gametophores. Some of the dwarf ♂♂ arose from protonemata whose origin was traced to large spores that had germinated in locations where they might have lodged in falling from an opened capsule. No ♂ gametophores appeared on other substrata. He concluded that the spores of this and other species, including one of *Schlotheimia* and one of *Trismegistia,* are differentiated as to size and as to sexual potentialities, the larger spores being ♂- determining and the species therefore dioecious.

Fleischer's conclusions have been questioned, partly on the ground that his "large, ♂- producing" spores may have swollen in the course of germination. Goebel (24) reported that only the larger spores of *Macromitrium* germinate, and that they do not give rise to dwarf ♂♂. The small, nongerminable spores, he concluded, are abortive. Schratz (cited by Correns, 16) found that measurements of the diameters of spores of *M. Blumei* (as well as of some other, including monoecious, mosses) yield a curve with two weak maxima. Dening (17), studying herbarium material of *M. Blumei* distributed by Fleischer, found in addition to the epiphytic dwarf ♂♂, small lateral ♂ branches borne by ♀ gametophores, the species being, then, monoecious.

The most extensive studies of *Macromitrium* are those of Ernst-Schwarzenbach (19–22). Spore-measurements, whether of the area of an optical section, of the diameter (of spherical spores),

or of the average diameter (of elongated spores), gave consistent
results. In some species, including *M. salakanum*, the measure-
ments yield a sharply bimodal curve, the mature spores falling into
2 approximately equal classes, large and small. These species are
classed as "heterosporous". In others, including *M. fasciculare,*
the curve is unimodal with narrow dispersion. These are "homo-
sporous". In *M. Blumei* the curve is indefinitely bi- to multi-
modal. This result is thus in general harmony with that of Schratz.
Ten of the homosporous species are clearly monoecious, a single
gametophore bearing both archegonia and antheridia. *M. Blumei,*
on the basis of Dening's study, is considered to be "polyoecious",
some gametophores bearing both kinds of sex organs and others
(dwarf ♂♂) bearing only antheridia. Seven homosporous and
20 heterosporous species are classed as "dioecious" in the sense that
they have separate ♀ (relatively large) and epiphytic dwarf ♂
gametophores. Strict dioecism is demonstrated only for *M. sala-
kanum*. In this, as in some other heterosporous species, the large
spores are green, the small spores yellow.

Protonemata from small spores of *M. salakanum* produced
dwarf ♂♂; secondary protonemata from these likewise gave rise
to dwarf ♂♂. Protonemata from large spores produced larger shoots
resembling in structure ♀ gametophores. A few of these produced
archegonia. Secondary protonemata from leaves of large shoots
gave rise invariably to large shoots. It is concluded that in this
species, and presumably in others resembling it, ♀ and ♂ potentiali-
ties are separated in spores, protonemata and gametophores.

Much discussed in this connection are species of *Buxbaumia,*
particularly the widespread *B. aphylla*. The ♂ gametophore con-
sists of a filament of cells bearing usually a single leaf and a single
antheridium. The ♀ gametophore, while small, is more massive,
bearing a few to 20 leaves and 1–5 archegonia. Dening (17) re-
ports that secondary protonemata coming by regeneration from ♂
gametophores produced only ♂♂, and secondary protonemata from
♀♀ produced only ♀♀. This species, then, is dioecious.

To test the question of heterospory, in the sense of a difference in
size between ♀- and ♂-determining spores, Dening measured spore
diameters in *Bryum caespiticium, B. argenteum, Ceratodon pur-
pureus, Mnium hornum* and *Buxbaumia aphylla*, all demonstrably
dioecious; in *Diphyscium foliosum, Dicranum scoparium* and *Pogo-*

natum aloides (see also 59), doubtfully dioecious; and in *Funaria hygrometrica,* monoecious. The spore-measurements in all species gave a unimodal curve.

SEX CHROMOSOMES

Lists of dioecious bryophytes in which sex chromosomes had been recognized were published by Lorbeer (45) and Tinney (96). Since a considerable number of additional cases have been reported in the past decade, it seems desirable to present a list brought, as nearly as possible, to date. Table 1 lists those dioecious species in which sex chromosomes, recognized by differences in size and often in form, have been positively reported. In Table 2 are given those in which observation has disclosed no differences between corresponding chromosomes of the 2 sexes.

In all reported cases of sex chromosomes in bryophytes except some species of *Frullania* (see below), the ♀ possesses a single X chromosome, the ♂ a single Y. The X is larger than the Y except in *Lunularia cruciata* and *Oxymitra (Tesselina) pyramidata,* where the Y is said to be the larger.

The sex chromosomes are in most cases reported to be heteropycnotic (heterochromatic), in the sense that the whole chromosome or a considerable portion of it remains dense and deeply stained at stages (*e.g.,* prophases) at which some or all autosomes are in large part diffuse and lightly stained. This statement holds especially for the X chromosome. Observations (and probably facts) differ as to heteropycnosis in the Y. Some of the autosomes in various species, apart from those mentioned in the next paragraph, are described also as having heteropycnotic regions.

In many bryophytes, both hepatics and mosses, and in both dioecists and monoecists, one chromosome is smaller than any of the others, frequently so small that it is easily overlooked. This has been designated an "m chromosome" (29). In some mosses assumed to be diploid or triploid because of their relatively high chromosome numbers, Heitz (32) found either 2 or 3 m chromosomes. Less often there is an "M chromosome" (2 in ♀♀ of some *Frullania* species) which is noticeably larger than any of the others. A particular species may possess an m, an M, or both. Chromosomes of both these types are frequently heteropycnotic. In many cases a sex chromosome is an m or an M.

TABLE 1

DIOECIOUS BRYOPHYTES: SEX CHROMOSOMES REPORTED PRESENT

SPHAEROCARPALES
    Riella helicophylla (Bory & Mont.) Mont. Lorbeer (44, 45)
    Sphaerocarpos cristatus Howe Allen (8)
    S. Donnellii Aust. Allen (1, 2),
                    Wolfson (106),
                    Lorbeer (44)
    S. Michelii Bell.
      (*S. terrestris* Smith) Lorbeer (44)
    S. texanus Aust.
      (Includes *S. californicus* Aust.,
      S. europaeus Lorbeer) Schacke (57),
                    Wolfson (107),
                    Lorbeer (44)

MARCHANTIALES
    Conocephalum supradecompositum
      (Lindb.) Steph. Tatuno (88, 95)
    Lunularia cruciata (L.) Dum. Lorbeer (45)
    Marchantia diptera Mont. Tatuno (84, 95)
    M. "grisea" Haupt (26)
    M. polymorpha L. Haupt (25),
                    Lorbeer (45),
                    Tatuno (84, 95)
    Oxymitra pyramidata Hüben.
      (*Tesselina pyramidata* Dum.) Lorbeer (45)
    Riccia Bischoffii Hüben. Lorbeer (45)
    R. Curtisii (Lindb.) T. P. James
      (*Thallocarpus Curtisii* Lindb.) Lorbeer (45),
                    Siler (71)

JUNGERMANNIALES ANACROGYNAE
    Calobryum rotundifolium (Mitt.)
      Schffn. Tatuno (73, 74, 95)
    Moerckia Blyttii (Moerck) Brockm. Lorbeer (45)
    M. Flotowiana (Nees) Schiffn. Lorbeer (45)
    Pallavicinia longispina Steph. Tatuno (73, 74, 95)
    P. Lyellii (Hook.) S. F. Gray Tatuno (75, 79, 95)
    P. radiculosa (Sande) Schiffn. Lorbeer (45)
    Pellia Fabbroniana Raddi Heitz (30, 31),
                    Tatuno (73, 75, 79),
                    Lorbeer (45)
    P. Neesiana (Gottsche) Limpr. Showalter (69, 70),
                    Heitz (32),
                    Tatuno (73, 75, 79),
                    Lorbeer (45)
    Riccardia (Aneura) blasioides
      Horikawa Tatuno (76, 79, 95)
    R. Makinoana (Steph.) Tatuno (92, 95)
    R. pellioides Horikawa Tatuno (83, 95)
    R. pinguis (L.) S. F. Gray Tatuno (76, 79, 95)

JUNGERMANNIALES ACROGYNAE
    Calypogeia suecica (Arn. & Perss.)
      K. Müll. Lorbeer (45)
    Frullania dilatata (L.) Dum. Lorbeer (45),
                    Tatuno (82, 83, 95)

TABLE 1—(Continued)

F. Fauriana Steph.	Tatuno (81, 95)
F. japonica Sande	Tatuno (81, 95)
F. nishiyamensis Steph.	Tatuno (88, 95)
F. sakawana Steph.	Tatuno (88)
F. squarrosa (R. Bl. & N.) Dum.	Tatuno (89, 95)
F. viridis Horikawa	Tatuno (83, 95)
F. yakushimensis Horikawa	Tatuno (83, 95)
Plagiochila asplenioides (L.) Dum.	Lorbeer (45)

ANTHOCEROTALES

Aspiromitus sampalocensis (Burgeff)	Rink (56)
A. spec. "Singapore"	Rink (56)

BRYALES

Ceratodon purpureus (Hedw.) Brid.	Heitz (33), Shimotomai and Kimura (63, 64), Jachimsky (35)
Mnium punctatum (L.) Hedw.	Jachimsky (35)[1]
Pogonatum grandifolium (Lindb.) Jaeger	Kurita (43)
P. inflexum Lindb.	Shimotomai and Koyama (65, 66)
P. spinulosum Mitt.	Kurita (43)
Polytrichum formosum Hedw. (P. attenuatum Menz.)	Shimotomai and Kimura (63, 64)

[1] Jachimsky finds a probably but not certainly unequal (XY) pair also in Mnium hornum L., Bryum argenteum L. and B. capillare L.

In all Sphaerocarpales thus far investigated, the Y is an m chromosome. In Sphaerocarpos cristatus and Riella helicophylla, the X, while much larger than the Y, is no larger than some of the autosomes—therefore not to be classed as an M. In all other species of Sphaerocarpos studied, the X is an M chromosome.

Haynes (27) concluded that S. texanus and S. californicus, both named by Austin, should be combined, the name texanus having priority. Lorbeer (45) has attempted to distinguish between the American and European forms referred to this species on the grounds of their incompatibility and of a difference in the structure of the X chromosome. The distinctions are based apparently on the study of a single strain from each source, and are obviously inadequate as taxonomic criteria. On the other hand, an examination of spores from various parts of the United States indicates that texanus is really a complex, ultimately to be divided into two or or more varieties or species. From Reimers' (55) study, it appears that similar differences occur among European races. For pres-

TABLE 2

DIOECIOUS BRYOPHYTES: RECOGNIZABLE SEX CHROMOSOMES REPORTED
NOT PRESENT

MARCHANTIALES
    Conocephalum conicum (L.) Dum.
      (*Fegatella conica* Corda)   Showalter (67),
                                    Lorbeer (45),
                                    Tatuno (86, 95)

    Marchantia cuneiloba Steph.   Tatuno (84, 95)
    M. radiata Horikawa   Tatuno (84, 95)
    M. tosana Steph.   Tatuno (84, 95)
    Riccia Curtisii (Lindb.) T. P. James
      (*Thallocarpus Curtisii* Lindb.)   McAllister (51)

JUNGERMANNIALES ANACROGYNAE
    Blasia pusilla L.   Lorbeer (45)
    Cavicularia densa Steph.   Tatuno (95)
    Haplomitrium Hookeri (Sm.) Nees Heitz (32),
                                      Lorbeer (45)
    Makinoa crispata (Steph.) Miyake Tatuno (86, 87, 95)
    Moerckia hibernica (Hook.) Gottsche Lorbeer (45)
    Petalophyllum Ralfsii (Wils.) Gottsche Lorbeer (45)
    Riccardia pinguis (L.) S. F. Gray
      (*Aneura pinguis* (L.) Dum.)   Showalter (68),
                                    Lorbeer (45)

JUNGERMANNIALES ACROGYNAE
    Alicularia scalaris (Schrad.) Corda Lorbeer (45)
    Chandonanthus birmensis Steph.   Tatuno (90)
    Eucalyx ovicalyx (Steph.) Horikawa Tatuno (77, 95)
    Frullania aoshimensis Horikawa   Tatuno (85, 95)
    F. densiloba Steph.   Tatuno (85, 95)
    F. Makinoana Steph.   Tatuno (85, 95)
    F. moniliata (R. Bl. & N.) Mont.   Tatuno (82, 85, 95)
    F. Tamarisci (L.) Dum.   Lorbeer (45)
    Leptoscyphus verrucosus (Lindb.)
      K. Müll.   Tatuno (91, 95)
    Madotheca (*Porella*) *densifolia* Steph. Tatuno (87, 95)
    M. japonica Sande   Tatuno (87, 95)
    M. parvistipula Steph.   Tatuno (92, 95)
    M. Perrottetiana Mont.   Tatuno (92, 95)
    M. tosana Steph.   Tatuno (92, 95)
    M. ulophylla Steph.   Tatuno (87, 95)
    M. ulophylla var. "*sacculosa*" Horikawa Tatuno (87, 95)
    Odontoschisma denudatum (Mart.) Dum. Tatuno (91, 95)
    Plagiochila adiantoides (Swartz) Lindb. Johnson (36)
    Pleurozia giganteoides Horikawa   Tatuno (95)
    Porella: see *Madotheca*
    Radula Lindbergiana Gottsche   Lorbeer (45)
    Schiffneria viridis Steph.   Tatuno (95)

BRYALES
    Barbula unguiculata (Huds.) Hedw. Jachimsky (35)
    Bryum caespiticium L.   Jachimsky (35), Wettstein
                                    and Straub (100)
    Mnium Maximowiczii Lindb.   Shimotomai and Koyama
                                  (65, 66)
    ²*Pogonatum aloides* (Hedw.) Palis. Jachimsky (35)

TABLE 2—(*Continued*)

[2]*P. subrotundum* (Huds.) Lindb.	
(*P. nanum* (Schreb.) Palis.)	Jachimsky (35)
P. urnigerum (L.) Palis.	Jachimsky (35), Kurita (43)
Polytrichum commune Hedw.	Jachimsky (35)
P. juniperinum Hedw.	Jachimsky (35), Kurita (43)
P. piliferum Hedw.	Jachimsky (35)
Rhizogonium Dozyanum Lac.	Kurita (43)

[2] If Schratz (59) proves correct in considering these two species monoecious, they, and possibly also *P. urnigerum*, should not be included here.

ent purposes, all members of this complex are treated as *texanus*. All that have been studied cytologically have similar sex-chromosome complements.

In all cases of sexually differentiated chromosomes reported in Marchantiales, except in *Riccia Curtisii*, both X and Y are m chromosomes. In *R. Curtisii*, McAllister found no differentiated sex chromosomes; each sex has an m chromosome. Lorbeer and Siler find a very small Y in the ♂; the X of the ♀ is no larger than some of the autosomes. The discrepancy appears to be explained by the fact that two distinct forms which have been referred to this species occur in the southern United States (28, 58). A form apparently identical with one of these is found also in South Africa (18).

Among various races of *Marchantia polymorpha* studied by Haupt (25) are 3 forms found in nature and six "mutations" (apparently derived from "forma *typica*"). In some of these races she detects no certain difference between the m chromosomes of ♀ and ♂. Since, however, these chromosomes clearly correspond to the X and Y in races in which a difference is apparent, Haupt holds them also to be sex chromosomes. The same argument may hold for the m chromosomes of several tropical species regarding whose size differences she is noncommittal. A like suggestion has been made (45) as to the m chromosomes of *Conocephalum conicum*, and may be applied to the 3 dioecious species of *Marchantia* studied by Tatuno (Table 2) in which also no size differences could be recognized between the m chromosomes of ♀ and ♂.

In the anacrogynous Jungermanniales, except that the X is in all reported cases larger than the Y, no definite rule holds, even within

a single genus. In *Riccardia pellioides, R. blasioides* and *R. Maki-noana,* the X is the M chromosome. In *R. blasioides,* the Y is the smallest (m). In the other 2 species, the Y is apparently not the smallest. In *Pallavicinia Lyellii* and *P. Flotowiana,* X is not the largest and Y is not the smallest. In *P. radiculosa* both X and Y are m chromosomes.

In *Riccardia pinguis,* Showalter and Lorbeer found no recognizable sex chromosomes. Tatuno finds an X, one of the largest chromosomes, and a Y, smaller but not the smallest in the set. Probably the Japanese plants studied under the name by Tatuno are not the *R. pinguis* of the United States and Europe.

In *Calypogeia suecica* and *Plagiochila asplenioides,* of the acrogynous Jungermanniales, both X and Y are m chromosomes. The frullanias found to possess sex chromosomes, alone among bryophytes thus far investigated, have an X element consisting of two chromosomes (X_1 and X_2) and a single Y chromosome. X_1, X_2 and Y are much larger than any of the autosomes. In *F. ornithocephala,* all plants grown from spores remained sterile (45). Some, presumably ♀, had 3 large heteropycnotic chromosomes; others, presumably ♂, had 2.

In the dioecious frullanias in which sex chromosomes are not recognizable (Table 2), there is in each sex one heteropycnotic M chromosome and 1 heteropycnotic m, the latter not present in those species with 2 X's and a Y. The species with and those without differentiated sex chromosomes belong, respectively, to distinct subgenera.

Tatuno (80, 95) suggests that, since many monoecious hepatics possess heteropycnotic M or heteropycnotic m chromosomes or both, and since the X and Y chromosomes of dioecious species (commonly heteropycnotic) are sometimes M and sometimes m chromosomes, sex chromosomes may have originated from 2 sources. In those cases in which the X or the Y is not distinguishable by size from some of the autosomes, Tatuno's hypothesis might be supported by the assumption of changes in size resulting from translocation.

In the two dioecious Anthocerotales with X and Y, both are m chromosomes.

Among mosses, conditions vary much as they do among hepatics. In the 3 species of *Pogonatum* reported to have sex chromosomes, and in *Polytrichum formosum,* the X is an M, the Y an m chromo-

some. In *Ceratodon purpureus,* both are M chromosomes. In *Mnium punctatum,* both are m chromosomes.

Lorbeer (44, 45) has figured the X and Y as more or less widely separated at early metaphase of the first meiotic division in *Sphaerocarpos Donnellii,* the autosomes being still intimately paired. He concludes that there is never a close pairing of X and Y, and hence no possibility of a cross-over between them. Their regular passage to opposite poles is unexplained. A like condition is described also (46), involving the 2 X's and the Y of a triploid sporophyte. Similar "distance conjugation" is postulated (45) for the sex chromosomes of other species of *Sphaerocarpos* and for a pair of "wholly heterochromatic" chromosomes in each of a considerable number of hepatics and mosses. In the latter cases the evidence consists in figures showing the bodies in question separated, while the members of each other pair are still in contact. Such figures, of course, may well result from an early separation of a closely conjugated pair. Distance conjugation is considered as related to the heterochromatic character of the chromosomes in question rather than to their function in sex-determination.

Heitz (34) criticizes this conception. He refers to various figures of other workers showing a close pairing of heteropycnotic sex chromosomes, as well as to certain of Lorbeer's own figures (44) which represent the X and Y as connected by a conspicuous strand.

Studies by Mrs. Duncan (unpublished) show that in *S. cristatus* there is a close pairing of the X and Y chromosomes in meiosis, "distance conjugation" here being clearly out of the question.

HETEROPLOIDY AND SEX

In *Sphaerocarpos Donnellii,* each spore of a dyad, or the large spore of a triad, commonly possesses, as shown by the gametophyte to which it gives rise, the diploid number $(2A + X + Y)$[3] present in the sporophyte. Gametophytes with this chromosome complement are in appearance and to some extent functionally ♀. However, they produce ocassional intersexual organs (6, 52), never reported in gametophytes without a Y. Lorbeer (46, 48) says that in the combination $2A + X + Y$, the Y disappears during germination of the spore, the X being later doubled and the

[3] "A" represents the haploid set of autosomes characteristic of the gametophyte. The numerical value of A varies with the species.

plant being finally ♀ with $2A + 2X$. That such changes are not of general occurrence is shown by Lorbeer's (44) own study of gametophytes with both X and Y, as well as by many observations in this laboratory. Knapp (39), in both X-irradiated and non-irradiated material, obtained, from dyad spores, $2A + X + Y$ gametophytes whose chromosome complement persisted without change. In a different category (37, 39) were gametophytes with $A + X + Y$ from irradiated spores which gave rise to ♀ branches with $A + X$ and to ♂ branches with $A + Y$. In gametophytes from dyad spores of *S. terrestris* (*S. Michelii*), "apomeiosis" having been induced, Lorbeer (48) finds that, contrary to what he thinks occurs in *S. Donnellii*, the full complement of $2A + X + Y$ persists.

In both irradiated and unirradiated material of *S. Donnellii*, while dyads from sporophytes developing between November and March gave rise only to the expected $2A + X + Y$ gametophytes (39), those from sporophytes developing between August and October produced predominantly 1 ♀ with $2A + 2X$ and 1 ♂ with $2A + 2Y$. Triads from these latter sporophytes yielded, when all spores germinated, either 2 ♀♀, 1 ♂ or 1 ♀, 2 ♂♂. Often of the 3 gametophytes from a triad, 2 were diploid, 1 haploid. In some instances all 3 were haploid. Apparently, seasonal conditions may influence the course of divisions in the spore mother cell. This may explain previously known occurrences of ♀♀ with $2A + 2X$ and ♂♂ with $2A + 2Y$ (7).

Matings of diploid × haploid *S. Donnellii* (5, 53) yielded triploid sporophytes $(3A + 2X + Y)$. The spores from only one family germinated in numbers. Of the tetrads giving complete germination, some yielded 2 ♀♀, 2 ♂♂, some 4 apparent ♀♀. Seven gametophytes survived for cytological study; 6 were apparent ♀♀ with $2A + X$, the possibility not being entirely excluded of an undiscovered Y in some; 1 was a ♂ with $2A + 2Y$. An apparent ♀ from a tetrad spore from another similar mating had $2A + X + Y$ (52). The failure of many spores to germinate and the early death of many gametophytes may well have been due to aneuploidy. Another mating of diploid × haploid with similar results is reported (39).

Mating of a diploid ♀ $(2A + X)$ with a diploid ♂ $(2A + 2Y)$ yielded a sporophyte producing spore tetrads (52). Each of 2 ♂ gametophytes from the spores had a chromosome complement of

2A + 2Y. Two ♀♀ had 2A + X; 1 apparent ♀ (producing inter-sexual organs) had 2A + X + Y.

The following chromosome complements are shown to be com-patible with viability in *S. Donnellii:* Gametophytic, ♀, A + X, 2A + X, 2A + 2X; intersexual, A + X + Y, 2A + X + Y; ♂, A + Y, 2A + 2Y. Sporophytic, 2A + X + Y, 3A + X + Y, 3A + X + 2Y, 3A + 2X + Y, 4A + X + 2Y, 4A + X + 3Y.

Dyad spores of *Marchantia polymorpha* and *M. alpestris* (15) yield ♀♀ (2A + 2X) and ♂♂ (2A + 2Y). Those of some tropi-cal marchantias produce in addition some gametophytes phenotypi-cally ♀, having presumably the equivalent of 2A + X + Y. Since in these tropical species X and Y can not certainly be recognized by differences in size (25), the small heteropycnotic chromosome in either sex is assumed to be functionally an X or a Y.

Of diploid gametophytes from spores of *Aspiromitus sampalocen-sis* (56), 28 produced antheridia. These ♂♂ had 2 Y chromosomes. The remaining 47 were presumed to be ♀♀, although only a few bore somewhat abnormal archegonia, usually but 1. Each of 2 producing archegonia had an X and a Y.

Regenerated diploid protonemata of *Splachnum rubrum* (10) produced only ♀ gametophores; but some of these bore paraphyses, typical of ♂♂. Of 2 diploid protonemata of *S. vasculosum,* similarly obtained, 1 produced only ♀ gametophores, of which 1 bore a paraphysis and 1 bore perichaetial leaves resembling those of a ♂. The other protonema produced many ♀ and a few ♂ gameto-phores.

Diploid monoecious gametophytes regenerated from the sporo-phytes of dioecious mosses are usually largely or wholly sterile. An instance has been described in *Bryum caespiticium* in which such sterility was overcome (98–100). The rare sporophytes borne by the regenerated gametophytes of this species produced mostly non-viable spores. The spores which germinated yielded chiefly diploid monoecious gametophytes similar to the parent plant; some gave rise to diploid ♀♀, diploid ♂♂, haploid ♀♀, haploid ♂♂, "monstrous" diploid monoecists, hypo- and hyperdip-loid monoecists, and sterile plants. One monoecious diplont be-haved differently from all others. In its first year it bore only antheridia, the next year and thereafter both antheridia and archegonia. Only embryonic sporophytes developed in the ear-

liest archegonia. In later years, normally developed sporophytes appeared; their number increased until in the course of 11 years the plant became fully fertile, producing viable spores all developing into diploid gametophytes of constant type. Gradually also the sizes of cells and organs diminished from those typical of *gigas* forms, finally reaching approximately the sizes characteristic of the original haploid parents. Portions separated from this diplont gave rise to clones which were similarly multiplied. Strains were grown also from spores, and these strains in turn were reproduced through spores. All clones and strains obtained in these ways were similar to the parent diplont, and all followed a parallel course of diminishing cell size and increasing fertility. The new constant, fertile diploid race is designated *B. Corrensii.*

Cytological study (100) showed that each of the 10 gametophytic chromosomes of typical *B. caespiticium* is duplicated in *B. Corrensii.* In meiosis in *B. Corrensii,* 20 chromosome pairs are present. There are no trivalent or quadrivalent groups, but evidences of secondary association of like pairs. In a wild diploid monoecious form of *B. caespiticium,* the meiotic behavior is similar. Wettstein thinks that the increase in fertility was a consequence of the diminution in cell size, this in turn being due to a dominant mutation. The exclusive formation of chromosome bivalents in meiosis in *B. Corrensii,* whereas the original diploid form derived by regeneration gave rise to sporophytes which, as indicated by the variance among their progeny, produced trivalents and quadrivalents, is explained by the occurrence in the spore giving rise to *B. Corrensii* of a genic combination which induces bivalent-formation. The appearance in nature of diploid races with cells similar in size to those of *B. Corrensii* suggests that gradual changes such as were observed in this race may occur in spontaneously produced diplonts.

<div align="center">INTRA- AND INTERSPECIFIC COMPATIBILITY</div>

Full compatibility is found between ♀♀ and ♂♂ of strains of *Sphaerocarpos Donnellii* from various localities (7, 9). The same is true as between all ♀♀ and ♂♂ within a single strain, and between ♀♀ and ♂♂ from spores of a single tetrad. The productivity of matings between typical and mutant clones seems to be limited only by the relative fertility or infertility of the mutants. Most attempted matings between plants of different species of

this genus have been unsuccessful. However, sporophytes have appeared (9) in crosses of *S. Donnellii* (♂) with 2 strains of *S. texanus* (♀). The spore tetrads produced by the hybrid sporophytes showed wall markings closely similar to those of the ♀ *texanus* parents. Most spores failed to germinate. A few from one sporophyte which germinated produced 3 mature gametophytes, 2 ♀♀, 1 ♂. These gametophytes were interfertile, and 1 ♀ and 1 ♂ yielded offspring in matings with clones of *S. Donnellii*.

A diploid ♀ (2A + 2X) *S. texanus* was crossed (39) with a haploid ♂ (A + Y) *S. Donnellii*. Among the gametophytic offspring were apparent ♀♀ which by elimination of an X chromosome produced ♂ branches, and intersexual and sterile types which changed to normal ♀♀ or ♂♂.

MUTATIONS INVOLVING SEXUAL CHARACTERS

In the previous review it was noted that (up to 1934) the problem of sex-determination in dioecious bryophytes appeared simple; by no means then discovered could a gametophyte of one sex be induced to manifest characters of the opposite sex. In species in which sex chromosomes could be identified, the presence of an X or of 2 X's implied femaleness; of a Y or of 2 Y's maleness; of an X and a Y, intersexuality or monoecism. More recent results suggest that the genetic situation may be somewhat more complicated than these statements imply.

Some cases of apparent sex-reversal already cited are explainable by the loss of an X or a Y chromosome. In the same category is a case (39) in which spore tetrads of triploid sporophytes of *Sphaerocarpos Donnellii* yielded in some cases 4 apparent ♀♀. Certain of these gametophytes produced ♂ branches. The explanation offered is that in a complement of 2A + X + Y the X chromosome was now and then eliminated.

From a mating of an apparently ♀ diplont from an interspecific *Marchantia* hybrid with a haploid ♂ of one parental species, a spore was obtained (15) which gave rise to a triploid ♂. From a mating of an apparently ♀ haplont (likewise hybrid and assumed to have the equivalent of an X and a Y) with a ♂ of a parental species, a diploid ♂ was obtained. In both cases it is suggested that either an X chromosome was lost or that it mutated to a Y. The occurrence of diploid ♀♀ derived from a mating of an apparently ♀ diplont (as-

sumed to have an X and a Y) with a ♂ diplont is explained by the mutation of a Y to an X. Male lobes appeared on diploid apparently ♀ gametophyptes derived from crosses involving several tropical species of *Marchantia*. Of such ♂ lobes, isolated, 22 developed into fully ♂ plants. Fourteen produced archegonia, 2 of them antheridia also. In addition, outgrowths combining ♀ and ♂ characters appeared on otherwise ♀ heads of *M. planiloba*. Cytological examination of plants developed from the aberrant ♂ lobes showed the full diploid number of chromosomes present. Burgeff suggests the mutation of an X to a Y chromosome. It may, however, be noted that the phenomena here are comparable to those in regularly monoecious bryophytes in which one gametophyte bears organs of both sexes variously distributed and may, as in several mosses, produce intersexual structures also.

More conclusive than these results with species of *Marchantia*, in which, incidentally, X and Y chromosomes are not certainly recognizable, are those from irradiation experiments with *Sphaerocarpos Donnellii*. Knapp's (37) X-irradiation of spore mother cells shortly before meiosis, or of young spore tetrads, resulted in the production from some tetrads of 1 ♀, 3 ♂♂ instead of the expected 2 ♀♀, 2 ♂♂. Tetrads of which only 3 spores germinated sometimes yielded 3 ♂♂. A few apparently similar results were obtained by irradiation of mature tetrads. When 3 ♂♂ arose from spores of a single tetrad, it was found that 1, sometimes distinguishable by greater size of cells and organs, possessed an X chromosome. In some instances, possibly in all, a variable portion of this chromosome had been lost. Evidently the sexual character of the spore had been reversed. In no instance was there a reversion from ♂ to ♀. Nor in any case save for a few tetrads from one sporophyte irradiated while still very young, were both of the potential ♀♀ of a tetrad changed to ♂♂. In these latter instances, Knapp suggests, radiation was effective while the X chromosome was functionally single. The ♂♂ resulting from reversion are sterile; their antherozoids are only weakly motile.

Lorbeer (47) X-rayed the growing tips of ♀♀. Later subjection of the plants to moisture and reduced illumination induced the appearance of regenerative shoots, of which a small minority were ♂. In one instance, the continued growth of the irradiated tip bore 2 longitudinal rows of antheridia and 1 row of archegonia. The

antheridia on the ♂ plants or parts so produced are somewhat larger than those on normal ♂♂. Irradiation of ♂♂ resulted in no change of sex (49); but sterile mutants were obtained by irradiation of either sex.

After irradiation of ♀♀ with A + 2X chromosomes, 3 monoecious gametophytes appeared which produced alternately ♀, ♂, and intersexual organs. In 1 of these mutants 1 X remained intact, the other was represented by a fragment. Occasionally the fragment disappeared in a cell or cells of the apical region, and the succeeding growth, containing only the intact "♂ X" (plus the autosomes), was ♂. A sporophyte resulting from a mating of this monoecious plant with a typical ♂ produced spores some of which gave rise to ♂♂ having the ♂ X and a Y. These gametophytes produced motile antherozoids, whereas the antherozoids borne by plants with a ♂ X and no Y were non-motile. The other 2 monoecious mutants were similar, except for translocations involving the ♂ X and some of the autosomes.

In *Riella helicophylla* a spontaneous mutation occurred in a haploid ♀ gametophyte (45, 47), which bore archegonia on its lower, antheridia on its upper part. The cells of the ♂ portion, including those of the antheridia, still possessed the ♀ chromosome complement (A + X). X-irradiation (49) induced a similar change of ♀ plants to the monoecious condition; in a few instances irradiation resulted in a change to complete maleness. All plants, whether ♀, monoecious, or ♂, possessed like chromosome complements. Similar experiments with ♀ *Marchantia polymorpha* yielded one monoecious and one ♂ plant, the latter with non-motile antherozoids. In these plants also the X chromosome persisted unchanged.

MUTATIONS INVOLVING VEGETATIVE CHARACTERS

Many structural mutations affecting other than sexual characters have been observed in *Sphaerocarpos Donnellii*. However, many of the mutants, whether spontaneous or induced, produce diminished numbers of sex organs, which are often largely non-functional. Typically, this, like other species of the genus, bears archegonia or antheridia profusely and continuously from a very early stage for as long as vegetative growth persists. *Tufted* strains (3) seem to be fully fertile. The *vegetative* race (9) bears a proportionally smaller number of sex organs and produces an

even smaller proportion of sporophytes. Vegetative ♂♂, however, seem fully interfertile with typical ♀♀. The *semisterile* character, known only in ♂♂, involves a still smaller proportion of sex organs. The *cupulate* character, also known only in ♂♂, involves a low degree of fertility. *Dwarf* ♂♂ have proved wholly infertile. *Sterile* males bear no antheridia. The *polycladous* race has fully fertile ♂♂, but the ♀♀, with only rare normal-appearing archegonia, are wholly sterile. So far as they have been studied, these mutants possess chromosome complements indistinguishable from those of the typical form of corresponding sex. This has been shown for semi-sterile and polycladous ♂♂, polycladous ♀♀ (106), dwarf and cupulate ♂♂ (52). A spontaneous sterile mutation is reported (47) in a ♀ clone.

Mutations showing structural aberrancies resulted from X-irradiation (37); their number was approximately proportional to the dosage. With equal dosage, the proportion of mutations was highest following irradiation of young spore tetrads shortly after meiosis, lowest when mature tetrads were irradiated, intermediate when radiation acted shortly before meiosis. One ♀ whose X chromosome lacked part of one arm (38) differed structurally from a typical ♀. When this was mated with a typical ♂, each tetrad of the resultant sporophyte gave rise to 2 ♀♀ aberrant like the mother and 2 typical ♂♂. The morphological mutation then is sex-linked. Of 16 mutations in ♀ gametophytes, not investigated cytologically, 9 proved similarly to be sex-linked; of 8 mutations in ♂ gametophytes, none were sex-linked. Some aberrant gametophytes (39) produced branches that approached the typical form—a change explained by the elimination of autosomes from a hyperhaploid complement.

Ten mutants (40), some spontaneous, some appearing after irradiation, were mated with typical gametophytes. As in the earlier results of the present writer (3, 4), some tetrads gave rise to but 2 genetic types of gametophytes in terms of sex and of the mutant character involved, some to 4 types. In matings involving each of 3 mutant characters, each tetrad gave rise to but 2 types; in those involving each of 7 mutant characters, differing proportions of tetrads produced 4 types each. Of 13 mutations which appeared (42) after exposure of antherozoids to ultra-violet light, 10 were lethal or sublethal.

By a- and X-irradiation of spores of *Physcomitrium piriforme* (11), over 100 mutant forms were obtained. Many of these, from the facts that only about 30% bore sex organs, and that only about half of these produced sporophytes, are assumed to be heteroploid. Those which proved fertile varied greatly in characters of protonema, gametophore, and sporophyte. The species is monoecious, antheridia being borne typically at the end of the main stem of the gametophore, archegonia at the end of a lateral branch. Some mutants bore both antheridia and archegonia in a single head.

GENETIC ANALYSIS

Knapp (40) concludes that the X chromosome of *Sphaerocarpos Donnellii* bears a gene or genes tending to femaleness. By irradiation such gene, or a sufficient proportion of such genes, may be inactivated, or may be lost with the elimination of the corresponding part of the chromosome. Since ♂♂ appear with the X chromosome thus modified and with no Y, a tendency to maleness is determined by a mechanism borne, not on the Y, but on some of the autosomes. Since the unmodified X is present only in the ♀, but a small portion of it at most being represented in the ♂ by the Y, Knapp argues that the greater part of the X lacks genes necessary to the existence of the gametophyte. This is further evidenced by the viability of occasional ♀♀ lacking various parts of the X. On the other hand, the fact that 9 of 16 radiation-induced structural mutations in ♀♀ proved to be sex-linked indicates that the genes involved in these mutations are borne on the X. This "totally heterochromatic" chromosome therefore carries genes which influence structure but are not essential to the life of the gametophyte. Genes involved in the 7 non-sex-linked mutations in ♀♀ must be borne on autosomes. Since all of 8 analyzed mutations in ♂♂ were non-sex-linked, there is no evidence that the Y chromosome carries genes affecting structure.

Linkage studies (41) established 4 groups (respectively 7, 2, 2 and 1) of genes presumably borne on 4 of the 7 autosomes. Knapp concludes that separation of the centromeres of each pair of homologous chromosomes occurs always in the first meiotic division, and that, as in other organisms studied, crossing over involves only 2 of the 4 chromatids of each chromosome pair.

Lorbeer (49, 50) postulates a ♀-tending gene (γ) on the X

chromosome of *S. Donnellii;* this is borne near the centromere, since it persists after the elimination of various parts of either arm of the chromosome. The Y chromosome bears a ♂-tending allel (*a*). The effects of irradiation upon sex expression show that a γ gene may be changed to σ, an intermediate allel for monoecism (*Riella, Marchantia*), or directly to an *a* allel (*Riella, Marchantia, Sphaerocarpos*). All sex-mutations thus far obtained are in the direction γ → σ → *a*. Elimination of portions of the X chromosome leads to subvitality or lethality unless the Y also is present. In the latter case, a deletion of part of the X is compensated. It follows that the Y possesses a complement of genes (except for γ) approximately equivalent to that borne by the X. The great difference in size between X and Y is explained by greater distances between genes located on the X. Determining for sex dimorphism is, in the ♂, the reaction system of the autosomes plus the *a* gene; in the ♀, probably the autosomal reaction system plus the γ and other genes in the X chromosome. Since ♂♂ resulting from irradiation, possessing a modified X and no Y, produce non-motile or very slightly motile antherozoids, the Y chromosome must bear a "mobilis" gene.

Reviewing previous work on polyploidy, Wettstein (99) points out that, while cell volume usually increases with the induction of auto- or allopolyploidy, the ratio of increase varies greatly in different polyploid combinations. In some allopolyploid series in mosses, cell size actually diminishes with an increase in number of genoms. It is concluded that the character of cell size in polyploid combinations is influenced by the interactions of parental genes as well as by the quantitative effect of an increase in chromosome number. A similar conclusion is reached by Barthelmess (12) on the basis of comparisons between haploid mutants of *Physcomitrium piriforme* and diploid races obtained by regeneration from the haplonts.

Discussing his earlier studies of interspecific and intergeneric moss hybrids, Wettstein (97) repeats his previously cited views (7) as to the genetic importance of the cytoplasm. Genom and plasmon, he emphasizes, are parts of the idioplasm, different in kind but equal in importance. Differences in cell size between reciprocal hybrids are ascribed to cytoplasmic influence (99).

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THE CYTOLOGY OF HOST–PARASITE
RELATIONS. II[1]

MABEL A. RICE
Wheelock College

This review is a follow-up of my discussion in The Botanical Review, September 1935, upon the physiology of parasitism with special reference to rust fungi. In that discussion the haustorium was considered an index of the high degree of adaptation effected by the parasite in that the increased feeding surface which it affords is gained, usually, without lysis of the host-cell walls or penetration of the primordial utricle and with a minimum of disturbance of host-cell metabolism. This adaptation is indicated by the general lack of chlorosis in the host tissue during the vegetative stages of the rust, and often by the actual retention of chlorophyll in infected areas as seen in the "green island" phenomenon. In contrast to this trend toward mutualism in a susceptible host the lack of adjustment in a resistant host is evidenced by chlorosis, altered metabolism and death of host cells, and by various stages of sheath formation in the parasite.

In the past decade the trend of work upon rusts has seemed to be toward cultural and biochemical rather than microscopic methods. Among the few studies which carry on the older tradition, Saville has presented a detailed microscopic study of nuclear structure and behavior in species of the Uredinales. She finds two distinct types of nuclei: the unexpanded migration nucleus of the vegetative mycelium including the haustorium, and the expanded form where the chromatin becomes distributed in an ectosphere. The latter are the nuclei concerned in spore formation (28).

McKenzie reports an experimental short cycle infection with *Cronartium quercuum*. He made successful infections of *Pinus sylvestris, Pinus banksiana* and *Pinus rigida* with true aeciospores from galls on *P. banksiana* and *P. rigida*. In his cytological studies he finds intercellular hyphae in the cortex and in rays of phloem and xylem. Haustoria are seldom found in young infections but are abundant in old infections. He interprets this difference in

the abundance of haustoria as evidence of a change in nutritional relations. He groups the host-plant reactions under three types: defoliation without permanent injury, death of leaders, and formation of galls. Comparing tissue from the first and second stages he found that in the first the rust mycelium had been cut off and checked by a cork layer, while in the second the mycelium had penetrated through the cork tissue into other living tissue where it remained alive for a time. It was this persisting mycelium which produced many haustoria. McKenzie concluded that the rust had not succeeded in attaining mutualistic relations with the host and that the transient infection indicated resistance of the host (17).

Thatcher made an interesting study of resistant-type flecks in Vernal wheat infected with race 34 of *Puccinia graminis tritici*. He made microscopic examination of the leaves from 18 hours after inoculation to the fifth day. On the fourth day, minute etiolate flecks were visible under a hand lens. The cells making up these flecks were dead or dying and contained degenerate chloroplasts, but they contained also well developed living haustoria. Even on the fifth day the entire mycelium of the fleck seemed alive. Thatcher concludes emphatically "that a fungus secretion leads to changes that cause death of the host cells some time before the parasite is itself injured" (35).

Dorothy Ashworth presents a detailed cytologic study of the life history of *Endophyllum sempervivi*. This history gives a picture of mutualism in a perennial rust. Spring infections by basidiospores into epidermal cells of leaves of *Sempervivum* become intercellular and haustorial, but, instead of producing spores, the mycelium grows down through the leaves into the cortex of the underground stem. It perennates here, but, since it does not during the first season approach the growing tip, new leaves at the centre of the rosette are free from disease to the end of that season. Renewed activity of the mycelium coincides, the next spring, with the growing season of the host. The mycelium now passes toward the growing point and into new leaves where sporulation follows. The mycelium in the infected leaves is thin-walled and has few haustoria. It shows marked growth in the direction of the long axis of the leaf but masses in the sub-stomatal spaces for sporulation. The mycelium of the underground stem is characterized by irregular form and by numerous stout haustoria (2).

This life history makes an interesting comparison with that of *Aecidium punctatum* Pers. to which I referred earlier (24), and *Endophyllum sempervivi* may be added to the company of *Aecidium punctatum* and *Chrysomyxa Pyrolae* as "outstanding examples of restraint on the part of the parasite toward the host in which they perennate" (24). *Aecidium punctatum* Pers. is perennial in roots and rootstocks of *Hepatica acutiloba*. It develops in the spring leaves while they are still in the bud; when they first appear above ground, the leaves are erumpent with spermogonia. These infected leaves develop into stiff reduced blades set on abnormally long petioles. Aecidia quickly follow the spermogonia and develop thickly on both leaf surfaces. By the end of May the infected leaves are dead. This result of heavy sporulation is in sharp contrast to the long life of uninfected leaves. The latter are active throughout the summer, supplying material for the over-wintering buds, and they themselves over-winter. In the rusted plants the photosynthetic work is carried out by a second crop of leaves in which the rust does not develop. Like the rust in the underground stem of *Sempervivum*, that in the underground parts of *Hepatica* remains dormant until the following spring. Thus an infected *Hepatica* plant in May will have three sets of leaves: reddened, overwintered, uninfected, lower leaves; infected, early spring leaves which, because of their hypertrophy, stand highest; and the tender, green, uninfected second-crop leaves of the spring. Rust-infected *Hepatica* plants have persisted for years in my woodland garden in vigorous condition, although with reduced blooming. The perennial mycelium in the rootstock occurs in cortex, in bundle sheath and in both ray cells and vessels of the xylem. In addition, it occurs in cortex, sheath and vessels of the wiry adventitious roots. In the roots haustoria are scarce, but intracellular hyphae penetrate the vessels and the cells of the bundle sheath. They often fill the cells of the bundle sheath, with branching runners, penetrating from cell to cell for long distances. The hyphal cells are larger in diameter and thicker walled than those of the leaves. In the rootstock the intercellular hyphae are frequently heavily encased in a brown-staining substance which is distinguishable from the host-cell wall. We note that Thatcher, investigating Kubanka wheat infected with *Puccinia graminis tritici,* concluded from microchemical tests that the enveloping material of the encysted haus-

toria was of fungal origin (35). The haustoria of the rootstock in *Hepatica* are also more or less heavily encased and range from small buttons completely encased to huge convoluted knots with lobes projecting from the sheath. These sheathed haustoria make a striking contrast to the numerous thin-walled haustoria in the *Hepatica* leaves where there is every indication of healthy growth of the rust in a healthy host cell. In the *Hepatica* leaf the rust practices a restrained feeding which breaks down only at the incidence of sporulation; in the rootstock there is evidence of lack of adjustment, but the rust makes luxuriant growth and is able to persist and grow into new tissues coincident with the awakening of the host plant to renewed activity in the spring. This again is like the habit of *Endophyllum sempervivi*.

An instance of heavy encasement attendant upon unsuccessful growth of a fungus is given by Bache-Wiig for the bracken parasite, *Cryptomycina Pteridis*. Where conidia had failed to penetrate epidermal cell walls the cell wall had become thick and dark in color below the appressorium. Even after penetration into the epidermal cell the fungus is, at times, confined to the single cell by a thick dark sheath formed around it (3).

A. M. Brown has made observations upon the perennial rust, *Puccinia minussensis* on *Lactuca pulchella*. He reports that "The perennial mycelium seems to alternate from the binucleate to the uninucleate condition and vice versa, depending apparently on the maturity of the host tissue involved and the food supply available to the fungus" (4).

Color changes in the leaves of infected plants seem evidences of altered metabolic reactions in the host cells and, when extreme, may be interpreted in terms of resistance. The following reports are given in order of increasing severity of the disturbance. Ruehle, reporting *Uredo sapotae* for the first time in the United States, notes that the fungus produces red-purple spots on leaves of *Sapodilla* but does not cause serious defoliation (27). Presley and King, describing *Puccinia stakmanii* n. sp. on cotton, note that anthocyan is produced by the leaf around the pycnial cluster (23). Arora, in a study of some rusts of Allahabad, notes that the mycelia caused disappearance of chlorophyll and disorganization of the host cells (1). An extreme case outside the rust group is reported by McDonough for *Sclerospora graminicola* on seedlings of *Selaria*.

The parasite early became systemic in seedlings. It resulted in stunting of the seedlings and in necroses of cells not even in contact with hyphae (16).

Hart and Allison were the first to report upon the browning reaction of Kubanka wheat when infected, at high temperatures, by certain races of *Puccinia graminis tritici*. This was not only a reaction of the host tissues, but seemed due, at least partly, to an encystment by a brown cell-wall-like deposit of many of the rust haustoria. Hart and Allison thought that the structures represented attempts at spore formation (11). Thatcher is more interested in the browning of Kubanka wheat as evidence of a change from susceptibility to resistance on the part of the host. In an exhaustive series of studies of osmotic and permeability relations in parasitism, Thatcher uses changes in host-cell permeability as an index of degrees of parasitism, since it indicates degrees of successful feeding by the parasite. In all cases, both for rusts and for a variety of other fungi, he found that the osmotic pressure of the cells of the parasite where higher than that of their respective host cells and that permeability increase is an effect of tissue invasion by a fungus in its respective host. For example, he found that *Puccinia graminis tritici*, race 21, caused an increase in permeability to cells of the susceptible wheat varieties, Mindum and Little Club, while resistance of Mindum wheat to race 36 was associated with a local decrease of host-cell permeability (34). Thatcher applied his microchemical tests for permeability to successive stages in the browning of Kubanka wheat and concluded that the browning "is an expression of a change in the micro-environment of the fungus induced essentially by high temperature which so modifies the interaction between host and parasite that the host reaction changes from complete susceptibility to a type of resistance leading to a cessation of fungal growth". By this and five other microchemical examinations of infected cereal tissues Thatcher concludes that permeability decrease is one factor tending to confer resistance; and that the delicacy with which the determining factors are balanced seems to indicate that some physiological factor such as a fungus enzyme may effect changes in the host-cell membrane (35).

Humphrey and Dufrenoy are also attacking the problem of host-parasite relations by means of cytochemical reactions. From their

microchemical tests upon leaves of oat plants infected with crown rust they believe that extremes of response to infection as well as all intermediate types of reaction can be interpreted cytochemically. From the living cells of a susceptible host, they state, the invading hyphae draw phosphorus compounds because of increased cell permeability; there is a secretion of vacuolar material out into the intercellular spaces, and phenolic materials are collected within the vacuoles into spherical bodies. In the resistant oats localized necrotic areas develop, and the mycelium, failing to make contact with living cells, induces a breakdown of nucleoproteins. The phenolic compounds yield deep-colored quinoid derivatives which "fix" constituents of the tissues around the infection area (13).

Alteration of cell contents in infected tissues has been reported for telial galls of *Gymnosporangium juniperi-virginianae*. Smits and Peterson finds a much higher concentration of carotene in the galls than in the leaves of the host. They state that the gamma isomer of carotene, rare in plants as far as they know, has not been found in as great concentration from other sources as in galls (29).

A variety of cultural experiments add data upon susceptibility and resistance. Gottlieb and Hart report negative results in testing the reaction to water-soluble growth substances of several varieties of wheat and oats under infection by physiological races of *Puccinia graminis tritici* and *Puccinia graminis avenae*. They conclude that water-soluble growth substances do not play an important role in the problem of resistance in cereal plants and that pathological differences in physiological strains of rust species may not be attributed to different growth substances (9).

Susceptibility with reference to the age of the host has been tested for *Cronartium ribicola*. Pierson and Buchanan, working with seedling of *Pinus strobus*, found that needles of the current season are low in susceptibility, needles of the second and third season are higher in susceptibility, and needles of the fourth season are of intermediate susceptibility. This ratio corresponds to the canker development, for the majority of cankers form on internodes of the second and third seasons (22).

Newton and Johnson tested the effects of temperature and light on the reactions to leaf rust, *Puccinia triticina* Erikss., of varieties

of wheat. They report that with a large number of races both temperature and light exercise a marked influence, with the more marked changes produced by variation of temperature (21).

Yarwood and Childs, testing the effects of rust infection on the dry weight of host tissues, found in ten out of eleven different rusts that the local dry weight per unit area was greater in rusted than in healthy tissue. In their summary we have a picture of the metabolism of a rust parasite. Rust infection apparently decreases the translocation of food from infected leaves because it interferes with photosynthesis; therefore less food is formed. It increases the respiration of infected tissues; therefore carbohydrates are used up. It causes fixation of dry weight in the infected tissue, as the rust fungi seem to be nourished principally by the labile organic materials of photosynthesis (37).

In this same connection comes a study by H. C. Murphy upon the effect of crown rust on the composition of oats. Sugars were noticably decreased in infected plants. Murphy explains this as due not only to the use of the photosynthate by the rust but to interference with the act of photosynthesis by broken epidermis, obstruction of the flow of liquids, chlorosis and necrosis, and shading of the host cells (19).

If, as I have suggested, "specialization is a corollary to obligateness" (24), the continuing reports of the determination of new physiologic races of rusts is the conclusive evidence of their adaptive powers. *Gymnosporangium globosum* Farl. might stand as an example of little race specialization, since MacLachlan reports it as occurring on at least ten genera of Pomoideae and three species of *Juniperus* (15). Yet if economic demands should push the work perhaps many physiologic races of *Gymnosporangium globosum* Farl. might be isolated.

In the heteroecious rusts of *Uredinopsis* Faull reports less specialization in the case of aecidial hosts than in the telial hosts. There is incidental evidence, he writes, that most species of *Abies* are more or less susceptible to all species of *Uredinopsis,* and all species of *Uredinopsis* may be passed to most species of *Abies,* while the species of *Uredinopsis* are closely restricted to their fern hosts (6).

Work done with the pine blister rust removes the ban from certain varieties of red currants. Hahn has demonstrated the im-

munity of the old varieties, Red Dutch and Viking, as against the common susceptible *Ribes sativum* (10). Snell reports that red currant varieties cannot be considered dangerous to white pine (30). Mielke reminds us of the shifting nature of resistance reactions by demonstrating that, although *Ribes lacustre* has lower susceptibility and lower telial production than three other species of *Ribes,* it is capable of rapidly intensifying blister rust on western white pine (18).

Puccinia asparagi offers another illustration of shifting resistance reactions. Its excessive virulence for asparagus crops has been held in check in recent years by the use of the resistant Martha and Mary Washington varieties of *Asparagus.* However, Fulton reminds us that the Washington varieties of *Asparagus* are resistant but not immune to *Puccinia asparagi* and reports that *Puccinia asparagi* is still doing considerable damage in Illinois; that it reached a peak of severity in 1940 to 1941. He relates this to an unusual abundance of inoculum, favorable environmental conditions and the absence of the rust parasite, *Darluca filum* (7).

Crown rust has come in for increasing notice in recent years. Murphy *et al.* report that the reduction of oat yields in Iowa from crown rust, in the period from 1919 to 1938, was more than twice that caused by the stem rust *Puccinia graminis avenae* Eriks. & Henn. This difference is laid to earlier work in distributing oat varieties resistant to stem rust and emphasizes the importance of breeding for resistance to crown rust (20). Rosen and Weetman report that with fall plantings of oats the varieties susceptible to and infected by crown rust gave a reduction in grain yields and sustained more severe frost injury than resistant varieties (26). Rosen also reports over-wintering of the uredinial stage of crown rust of oats in Arkansas in 1941–1942, but has not determined whether mycelium or uredinial primordia were responsible (25). Buchholtz reports that the 1941 epiphytotics of *Puccinia rubigovera* and *Puccinia coronata* in South Dakota were the worst known thus far in eastern South Dakota (5).

Much work has been done also with stem rust of oats. Levine and Smith consider the physiologic specialization of *Puccinia graminis avenae* in the United States as restricted, but in a comparative study of the reaction of seedling and maturing stages of oats to the rust they inoculated 10 physiologic races of the rust on

27 varieties of oats. They concluded that the seedling reaction is a reliable index of the reaction of the adult oat plants to the races of the rust (14). Tervet and Hart report a variation in the reaction of Anthony oats to race 5 of *Puccinia graminis avenae*. They point out that selection within a variety is an excellent means of obtaining rust-resistant strains and that probably many disease-resistant crop plants need periodic re-examination, exposure to artificial epidemics, and re-selection within the variety to ensure the maintenance of resistance (33). Stakman and Loegering point to race 8 as a possible danger to resistant oat races of Victoria × Richland crosses. They conclude: "It is clear that there have been decided and important changes in the prevalence of races of *Puccinia graminis tritici* and equally great changes may or may not occur in *Puccinia graminis avenae*" (31). This reminds one of the reference by Thatcher to the delicacy with which the determining factors are balanced between the diverse manifestation of resistance and susceptibility to rusts (35).

The work with *Puccinia graminis tritici* in breeding for rust resistance still leads, since here one is dealing with danger to the staff of life. In 1935 I quoted Humphrey who gave the total number of physiologic races of *Puccinia graminis tritici* to date as "something like 152 (24). In 1942 Stakman *et al.*, in announcing an unusually virulent race of wheat stem rust collected from Peru, numbered it 189 in the Stakman and Levine key. A quotation from their report brings us back again to the problem of the nature of resistance and susceptibility. "The existence of so virulent a race as 189 is definite confirmation of the statement so often made in the past that nature can produce very virulent races of rust just as man can produce very valuable varieties of wheat". . . . "Therefore it is important to study the prevalence and distribution of physiologic races, eradicate the alternate host when feasible and continue breeding work to find and combine genes for resistance in the most effective manner possible". Again, Stakman emphasizes variability: "A variety of wheat may be resistant in one region and susceptible in another in the same year. A variety may behave differently in the same place in different years. Changes may be gradual or sudden. There is little definite information regarding causes for changes in previous years although seasonal temperatures, distribution of wheat varieties and conditions affecting winter survival of the fungus are pertinent" (32).

The importance of breeding for rust resistance is well illustrated by the history of work with bean rust. Wingard's pioneer experiments in the development of rust-resistant beans by hybridization (36) turned attention to this rust. Harter and Zaumener state that in 1935 two races of bean rust had been differentiated. Now they have announced the differentiation of 20 physiologic races of *Uromyces phasioli typica*. These were established on the basis of the size of a pustule at the end of 14 days on seven different host varieties from the United States and Hawaii (12).

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THE BOTANICAL REVIEW

THE PROPER DESIGNATION OF THE VASCULAR PLANTS

THEODOR JUST

University of Notre Dame

The status and growth of taxonomic, morphological and paleonto-logical knowledge of the plant kingdom are to a large extent reflected in the various classifications adopted from time to time. Consequently new advances and viewpoints are likely to result in proposals for certain rearrangements or new alliances which are often accompanied by new designations. Thus it is not at all surprising that in recent years several new names have been proposed for the higher taxonomic unit comprising all vascular plants. These proposals were made despite the fact that in the past the vascular plants had repeatedly been treated as a group. On the other hand, these new names indicate clearly the present revived and increased interest, whether morphological, genetical or otherwise, on the part of botanists in the sporophyte of vascular plants. As a result, a great deal of morphological and anatomical knowledge is now being reexamined and evaluated from many new points of view. This renewed interest is, then, the direct outgrowth of the many remarkable advances made in morphology, paleobotany, genetics and other fields.

It seems desirable, therefore, to consider at this juncture the more important designations proposed for the vascular plants together with the criteria and evidence claimed in their support. Curiously enough, several of these designations were not published in conformity with the International Rules of Botanical Nomenclature and thus must be regarded as *nomina nuda* (see especially Recommendation viii and Article 37). Without attempting an exhaustive treatment of all designations published since the beginning of modern botany (Winckler, 1854, du Mortier, 1864, Gmelin, 1867), the following discussion should illustrate the difficulties attending the selection of a suitable name in this as well as similar cases and

the desirability of having the entire problem of the names of higher categories presented at the next International Botanical Congress. Strict application of the Rules to many widely used names of higher categories, old or new, will inevitably result in a long list of *nomina conservanda*.

Apparently A. P. De Candolle was the first botanist who grouped all vascular plants together as "végétaux vasculaires" (1805)[1] and referred the others to "végétaux cellulaires". The name "Plantae Vasculares", proposed by him for this group in 1818, has as such, or abbreviated to "Vasculares", been widely adopted in floras, manuals and other systematic treatises. However, dissatisfied with his own disposition of the ferns and fern allies, he removed them later (1833) from the Monocotyledoneae among the vascular plants and erected for them the new group "Semi-vasculares", which in turn was relegated to a place among the cryptogams or cellular plants. Noteworthy in this connection is also the opening statement of the Latin diagnosis (De Candolle 1818, p. 121) which reads as follows: "Plantae contextu celluloso vasisque lymphaticis constantes; stomatibus instructae"; *etc.* De Candolle was firmly convinced that this classification (1833) was correct, because the study of vegetative (vascular *vs.* cellular plants) as well as reproductive organs (Cotyledoneae *vs.* Acotyledoneae) had led him to distinguish equivalent groups. Although nomenclaturially speaking, "Plantae Vasculares" is a valid name legitimately published, the somewhat ill-defined or often erroneous anatomical concepts of that time and the ideas of relationship held by its author are, in part at least, responsible for the appearance of many later designations.

In subsequent classifications, especially those following Hofmeister's classical researches, vascular plants appear as a part of a larger entity such as "Cormophyta", "Prothallatae", "Embryophyta", and "Archegoniatae." There can be no doubt that, morphologically speaking, the gap between the thallophytes and all other groups—here conveniently though inappropriately included in Schaffner's "Metathallophyta"—is probably the largest known of any major plant groups. For this reason the similarities of all higher plants appear much greater if viewed on the basis of their

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[1] Alph. De Candolle corrected the dates of publication of "Flore Française"; only the last volume was issued in 1815, whereas the others were published in 1805. See Ber. Deut. Bot. Ges. 7: 394, footnote 2, 1889.

homologies than if studied from some particular viewpoint and
certainly if compared with the numerous unrelated groups custom-
arily referred to the thallophytes. It should be remembered, how-
ever, that the term "Thallophyta", despite its wide use in textbooks,
is today outmoded, irrespective of the fact that it continues to be a
convenient term needed to indicate concisely a certain morphological
level. If this is recognized, "Thallophyta" can never be considered
cognate with natural groups which include only related forms.
Consequently, several authors have dropped "Thallophyta" as a
major group and substituted for it a number of independent phyla
(Bessey, Engler, Fritsch, Pascher, Schaffner, Smith, Wettstein,
et al.).

The metathallophytes, on the other hand, have been treated as a
larger unit or divided into separate phyla (Berry, Bessey, Eichler,
Engler, Haeckel, Schaffner, et al.). Disregarding here the now in-
adequate Linnaean distinction of "Cryptogamae" and "Phanero-
gamae", the problem involves mainly the nature of the evidence
supporting either the maintenance of two or more divisions or
phyla, or their inclusion in a major category. Thus phylogenetic or
other considerations have at different times led to the proposal of
very large divisions made up of thallophyte and metathallophyte
members or groups. The following examples may illustrate this
point.

Using the presence or absence of sexuality and of spore-produc-
ing structures as the principal criteria, MacMillan (1892) proposed
the term "Sporophyta" for all groups with sexual reproduction and
sporophytes (higher algae, fungi to Angiospermae). This usage
of "Sporophyta" obviously differs from the very opposite applica-
tion of it to all spore plants, formerly known as "Cryptogamia"
(Willkomm, 1854). Hallier (1905) used "Sporophyta" in still
another sense by restricting it to the pteridophytes. Needless to
say, little can be gained by such confusion.

Thallophytes and bryophytes also have been combined at different
times and for various reasons. For instance, Bugnon (1922)
called them "Cytophytes", or, like some older botanists, "Cellu-
laires". In the same year Gravis recommended replacement of the
terms "gametophyte" and "sporophyte" with "gametophore" and
"sporophore" and recognized two major groups, namely, "Gamé-
todynames" with dominant gametophytes (phycophytes and bryo-

phytes) and "Sporodynames" (pteridophytes and spermatophytes)
with dominant sporophytes. Our present knowledge of the life
histories of many lower plants certainly disposes readily of such
an attempt to utilize the relative development of gametophytes and
sporophytes as the sole criterion of classification of major groups.

Other attempts of combining thallophytes and bryophytes were
based largely on organographic evidence. Thus van Tieghem
(1906) called all plants without roots or vascular elements "Arhizo-
phytes" or "Invasculaires", whereas those plants which bear roots
and contain vascular tissue were designated "Rhizophytes"[2] or
"Vasculaires". Later Troll (1937) accepted the distinction of
rhizophytes and arhizophytes and pointed out that the morphology
of the root provided the only criterion for the typological character-
ization of the pteridophytes and spermatophytes. On this basis he
designated the former as "Homorhizophyta", whose roots arise as
lateral organs from the shoot, and the latter as "Allorhizophyta",
whose embryo has two growing points, one of which gives rise to
the primary root.

The worst professedly phylogenetic system proposed in recent
years is based solely on the presence or absence, the character and
number of flagella found on reproductive organs throughout the
plant kingdom. According to its authors, Sakisaka and Sinoto
(1930), phylum 4 or "Polycontophyta" apparently includes all
metathallophytes in strange new alliances. The last subphylum of
this phylum, the "Stephanokontae", made up of the Oedogoniales
and Derbesiales, apparently represents the climax of the plant king-
dom.

Many botanists are now agreed on dismembering the old sub-
kingdom Thallophyta, but are still in disagreement on treating the
metathallophytes as a natural entity or as two or more distinct phyla.
Unger (1838) was the first to contrast "Thallophyta" (plants with-
out axes) with "Cormophyta" (plants with axes). This dis-
tinction was immediately adopted by Endlicher (1840), although
the term "Cormophyta" has since been applied in various ways.
For instance, Alexander Braun (1864) as well as Arber (1921)
used it for the pteridophytes, and Haeckel (1894) for the vascular
plants, whereas Lotsy and Wettstein applied it to the entire meta-

[2] Rudolphi proposed the term "Rhizophyta" in 1830 and applied it to fungi
and lichens (fide Zunck, 1840). Van Tieghem's term is therefore a later
homonym.

thallophyte complex, as originally defined. As pointed out above, since more evidence can be presented in favor of the unity of the metathallophytes than that of the thallophytes, the term could well be applied to this subkingdom in Endlicher's sense and as redefined by Lotsy and Wettstein, if the other subkingdom is to be called "Thallophyta". Given this interpretation these terms would at least be cognate, because they would be based on the same criterion, *i.e.*, the structure of the plant body as a whole. Even without retaining "Thallophyta", "Cormophyta" should be used as the oldest available name for the metathallophyte complex (Wettstein, 1935) rather than in Haeckel's restricted sense for the vascular plants.

Engler's widely accepted term "Embryophyta" (1886) is, by comparison, based on the fact that in all metathallophytes zygotes invariably develop into multicellular embryos while confined in female sex organs and that the plants are capable of continued growth or continued embryology. However significant these characters may be, they pertain first of all to a stage in the life history of these plants and not to the plant as a whole; also their "continued" embryology (continuous presence of formative tissue) is more effective in the spermatophytes than in the pteridophytes (except the Psilophytales) and is absent from the bryophytes (Bower, 1930).

Similar arguments might be advanced with reference to the use of the term "Archegoniatae" s.l., applied as a synonym of "Embryophyta" by Conard (1919) and Torrey (1932). Another point would be that "Archegoniatae" s.s. has long been used for the combination Bryophyta and Pteridophyta (Engler, Bower, Wettstein, *et al.*) or for these two groups and the Gymnospermae (Kousnetzow, 1922), irrespective of Nilsson's (1941) efforts to reaffirm the archegoniate nature of angiosperms. Even those who place the bryophytes side by side with pteridophytes and spermatophytes admit the numerous similarities existing between bryophytes and pteridophytes. Surely "Archegoniatae" s.s., also called "Prothallophyta" (1866) and later "Diaphyta" (1894) by Haeckel, should be dropped only if the vascular plants deserve to be grouped together in preference to the classifications based mainly on gametophyte characters. Harder (1939) expresses this opinion clearly and states that, although no direct connections between bryophytes and pteridophytes are known and the latter are related to the sper-

matophytes (both have tracheids, etc.), a distinction into cellular *vs.* vascular plants in the De Candollean manner would obscure the relationships existing between bryophytes and pteridophytes.

After this account of the most commonly used designations of the entire metathallophyte complex, a discussion of the terms proposed for the vascular plants as a unit of varying extent is now in order. In addition to the terms and classifications discussed above, the following proposals are of interest here. Stolley (1925) removed the Psilophytales from the Pteridophyta and placed them below the Bryophyta with a rank equal to these two groups, the Pteridospermae, Gymnospermae and Angiospermae. The viewpoint expressed in this arrangement approaches that of Church (fide Brown, 1935) who regards the Rhyniaceae as primitive Bryophyta, whereas Arber (1921) is of the opinion that "Procormophyta" are "halfway between the Thallophyta and Pteridophyta". These dispositions of the Psilophytales failed to gain recognition and acceptance, however.

Pascher's (1931) term "Pterido-Anthophyta" is in reality no more than a compromise. Similarly, Haeckel's (1894) distinction of "Cellophyta" (= Bryophyta) and "Vasophyta" (= Pteridophyta and Spermatophyta) as well as Bugnon's (1922) distinction of "Gametophyteae" (Gamètophytées = Bryophyta) and "Sporophyteae" (Sporophytées = vascular plants) do full justice neither to the problem at hand nor to the newer knowledge of the lower plants. Such terms are at best misnomers. Conard's (1919) division of the "Embryophyta" into "Atracheata" (Bryophyta) and "Tracheata", which reflects Jeffrey's views, marks a great improvement over earlier proposals and was therefore adopted by Torrey (1932). Unfortunately these designations might easily be confused with names long in use in zoological classifications, irrespective of other considerations discussed below.

Van Tieghem's term (1906) for all vascular plants, "Rhizophyta", was restricted by Bugnon (1922) who removed the rootless members found among the earliest Psilophytales and placed them in the "Thalloxylophytes". Although certain living pteridophytes are also rootless, Troll (1937) claims that their embryo structure is such that, if roots were present, they would develop like those known in other pteridophyte forms. The reader is referred to Bower (1935) and Schopf (1943) for more detailed discussions of the "primitive spindle" and its significance in this connection.

Another designation, first published by Sinnott (1935) and later used by Eames (1936), Foster (1941), Tippo (1941), Darrah (1939, 1942), and MacDougall and Hegner (1943), is "Tracheophyta",[3] which is regarded as "cognate with Thallophyta and Brypophyta" and includes "the vascular plants as a whole." It is based on the appearance in vascular plants of "tracheary elements" and not primarily on the fact that the elaboration of the sporophyte was made possible by the development of the conducting system. Since tracheary elements are basic constituents of the xylem of all vascular plants, the more inclusive term "Xylophyta", though originally proposed by Clements (1902) for ecological usage of limited applicability, was actually applied to all vascular plants by Bugnon (1922) and Stefanoff (1937). These designations, *viz.,* "Tracheata", "Xylophyta" and "Tracheophyta", indicate the great importance now attributed to the tracheary elements in the evolution of the sporophyte of vascular plants, which is also well substantiated by the paleontological record (Hofmann, 1934).

One more term remains for consideration in this connection. Like others it too was proposed by its author in a rather casual manner. In his review of Zimmermann's well known book on the phylogeny of the plant kingdom (1930), Pia (1931) recommended a different treatment of the "Cormophyta" from that adopted by Zimmermann and suggested the term "Stelophyta" for the vascular plants.

Undoubtedly this term would be acceptable only as long as the stelar theory is held in high regard by botanists.[4] In Wardlaw's opinion (1944), the older botanists dealt with the *"individual strand* as the unit of vascular construction", whereas "van Tieghem (1886) recognized the *vascular system,* whether compact and simple or disintegrated and complex, as the unit. This he called the stele. It was a conception which, like one that had already been formulated by Sachs, tended towards a recognition of the essential unity of the shoot". Eames and MacDaniels (1925) assessed the influence of the stelar theory on botanical knowledge in the following way: "the establishment of the stelar theory has supplied a basis for an

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[3] Although this term seems to have been used long before it was published, it is apparently impossible at this time to determine the author of it (A. J. Eames in litt.).

[4] Moll (1934, footnote on p. 313) points out the different meanings which have been attributed to the word "theory" as used in the combination "stelar theory".

understanding of the structure of the plant body such as was not possible before. This alone has brought the vascular skeleton to the front as of much importance in the study of phylogeny". Granted that many objections to details of the stelar theory have been raised (Campbell, 1940; Watson, 1943; *et al.*) and disproved (Rudolph, 1921; Schoute, 1926, 1938; Smith, 1938), and the discussion of the nature and origin of stem, leaf and root has not yet completely subsided (Eames, 1935; Cappelletti, 1936; Bower, 1930, 1935; Zimmermann, 1930, 1938; Arber, 1941; Wetmore, 1943; Wardlaw, 1944), these conditions need not affect the possible acceptance of the term "Stelophyta" any more than similar situations have affected the use of other terms such as "Anthophyta", "Spermatophyta", "Spermaphyta", "Angiospermae", *etc.* In addition, Smith (1938) shows that the discovery of the most primitive land plants confirmed the essential postulates of the stelar theory long after its original pronouncement and subsequent elaboration, and Bower (1935) concludes that "the vascular tissues provide the most constant characters of the plant body".

It may not be entirely out of place to mention here that the phenomena of alternation of generations, sex determination and heterospory are currently being subjected to experimental studies and that new interpretations are gaining ground (Schiffner, 1925; André, 1938; Pincher, 1935, 1937; Thomson, 1934; *et al.*). Many supposedly well established concepts are bound to be replaced in this process. Some day perhaps even the architecture of the plant body and the morphogenetic processes bringing it into being will become clear in all their ramified complexity, since many primitive land plants are already fairly well understood (Zimmermann, 1938). At that time a new designation based on external morphological characters may again become desirable, but it should be better than Cohn's "Pteridophyta" (1872) which was never the best designation for the habit of the fern allies.

In conclusion it may be pointed out that, unless convincing proof of the valid status of one of the older designations can be furnished, botanists are apt to look for a new term. Unfortunately, according to the International Rules of Botanical Nomenclature, the more recently proposed designations, *viz.*, "Xylophyta", "Stelophyta" and "Tracheophyta", are *nomina nuda* which can not be used as such. Nevertheless, the last of these *i.e.*, "Tracheophyta", has gained

limited acceptance, predominantly in morphological literature. With few exceptions most designations of the higher category including all vascular plants are based on anatomical characters of varying significance, the most general term being "Stelophyta", whereas "Tracheophyta" is the most specialized of the group. The case of the proper designation of the vascular plants illustrates the great need for consistent application of the International Rules of Botanical Nomenclature to the names of higher categories and for a critical review of the most widely accepted terms by the Subsection of Nomenclature of future International Botanical Congresses.

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ABSORPTION OF WATER BY PLANTS

PAUL J. KRAMER
Duke University

INTRODUCTION

This review is concerned primarily with the nature and origin of the forces bringing about movement of water from soil or other media surrounding roots into the conducting elements of the xylem, and with those internal and external factors which affect the rate of such movement.

Absorption of water is not an independent process, but is closely related to other processes included in the domain of plant water relations. The rate of water intake is markedly affected by the rate of transpiration and by the extent and condition of the root systems. It is also affected by such environmental factors as the available moisture content of the soil, soil temperature, soil aeration, and to a lesser extent by the kind and concentration of ions in the soil.

Some phases of the work discussed in this review naturally are incomplete and do not permit formulation of definite conclusions concerning certain aspects of the absorption problem. Where the writer has drawn conclusions or made generalizations they seem to be those most justifiable on the basis of the available evidence. It is realized, however, that as knowledge of these processes increases it may be necessary to modify some of the present conclusions. It is impossible to cite all the literature, but most of the papers cited have bibliographies in which the reader can locate other papers dealing with any particular phase of the field.

ABSORPTION MECHANISMS

The Existence of Two Absorption Mechanisms

Intake of water by roots apparently is brought about by two quite different and independent mechanisms which probably do not even operate simultaneously. When soil moisture is abundant and transpiration is slow, absorption frequently exceeds water loss, resulting in the development of hydrostatic pressure or "root pressure" in the xylem. Since this type of absorption occurs only in plants with healthy roots, and according to some

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theories is dependent on the expenditure of energy by cells of the roots, it has been termed "active absorption" (212). The mechanism responsible for active absorption and the resulting guttation and exudation from cut stems will be discussed later.

The situation existing in the water-conducting system of rapidly transpiring plants appears to be quite different from that existing in well watered, slowly transpiring plants. During periods of deficient soil moisture or when the rate of transpiration is moderate to rapid no root pressure or exudation can be demonstrated. Instead, the pressure on the water in the xylem vessels is nearly always lower than atmospheric pressure, as evidenced by instantaneous penetration of liquids into incisions made in the xylem of such plants. Under such conditions the roots appear to act simply as passive absorbing surfaces for water, as evidenced by the fact that transpiring plants can absorb considerable water through dead roots (137). This type of water intake has therefore been termed "passive absorption" (212) to distinguish it from absorption which is dependent in some manner on the activity of living root cells.

The relative importance of active and of passive absorption has been warmly debated. After describing the two mechanisms evidence concerning their relative importance in growing plants will be presented.

Active Absorption and Root Pressure Phenomena

Species from which exudation occurs. Sap for beverages has been obtained for centuries from palms and agave, and the first settlers of New England found the Indians tapping sugar maples and boiling down the sap. Exudation from grapevines has also been known for many centuries, but according to Sachs (228), Hofmeister (112) was the first to demonstrate that exudation commonly occurs from the stumps of many cultivated herbaceous species. No recent attempts have been made to list the species exhibiting root pressure, but it certainly occurs in scores and probably in hundreds of species (54). Wieler (268) collected from the literature references to "bleeding" or "weeping" in 126 species belonging to 93 genera and 47 families, distributed among the ferns, gymnosperms and angiosperms, and added from his own observations 62 additional species. Many species are included

by Wieler which probably do not really exhibit root pressure, as
he apparently included exudation from glandular hairs, woody
tissue, and even from root systems previously soaked in salt
solution. Several conifers were listed, for example, but none of
them is known normally to exhibit true root pressure. It has
been produced artificially in certain coniferous species by first
soaking the root systems in salt solutions, then transferring them
to pure water (77, 268). It has not been determined whether
the absence of root pressure in various species is the result of
anatomical or of physiological conditions.

Types of exudation. Considerable confusion has resulted from
failure to distinguish between exudation caused by root pressure,
exudation caused by local stem pressure, and that caused by the
activities of special cells such as those of glandular hairs. Wieler
(268) seems to have treated all types of exudation of liquid as
examples of "bleeding", but Clark (42) and Sachs (228) had
already distinguished between exudation caused by root pressure
and exudation caused by pressure developed in the stems of woody
plants. It is probable, for example, that the exudation of sap
from a wounded grape or birch stem is caused by root pressure
because it occurs only after the soil has warmed up in the spring
and after the temperature remains continuously above freezing.
Furthermore, it continues rather steadily, day and night instead
of fluctuating with temperature as does maple sap flow (126).
Exudation of sap from the sugar maple, on the other hand, is
probably largely or entirely caused by local stem pressure re-
sulting from increasing temperature (42), though some investi-
gators believe that the activity of living cells must be involved
(126). Maple sap flow usually will occur any time in the winter
that a cold night is followed by a warmer day with temperatures
above freezing, and it ceases abruptly in the spring when wide
variations between day and night temperatures cease, just when
sap flow from birch and grape is beginning. By attaching
gauges to maple roots it was found that positive root pressures
were too infrequent and too slight to account for the flow of sap
from tapped trees (41, 42, 126). Molisch (188) regarded the
stem pressures and exudation reported in trees by Hartig and
Figdor as caused by activity of cells in the immediate vicinity
of the wood and not by root pressure. The flow of sap from

palms following removal of the inflorescence was also regarded by him as a local secretion because it is maintained only by repeated wounding, and, furthermore, because root pressure is not generally observed in the lower part of the stem of palms. Exudation from wounds in stems of cacti and Monterey pine are also said to be caused by the activity of living cells in the immediate vicinity of the wounds, and no root pressure was ever observed in pine (169, 170). There has been some debate concerning the occurrence of root pressure in aquatics, but Thut (247) reinvestigated this problem and found that measurable quantities of sap exuded from cut stems of several species of submerged aquatics, largely as the result of root pressure. It is generally agreed that most guttation is caused by root pressure, although drops of water are sometimes secreted from modified epidermal cells and multicellular hairs (91).

This review will be concerned primarily with exudation of liquid caused by pressure originating in the roots. Secretion by glands and exudation caused by local stem pressures will receive only incidental consideration.

Volume of exudate. The volume of exudate depends on the size and type of plant, the ratio of absorption to transpiration and on various environmental factors, especially temperature and available soil moisture. Sugar maples may yield five to six liters of sap in a single day and 25 to 75 liters in a season. Ironwood and birch are said to yield larger volumes of sap than sugar maple, a paper birch 37.5 cm. in diameter having produced about 28 liters of sap in one day and 675 liters during the season (42). The agave is also said to yield hundreds of liters of sap over a period of several weeks. Sachs (228) writes of a sunflower plant about three meters in height with a stem diameter of four to five centimeters which exuded 1,061 ml. of sap from the stump in 14 days, or a volume of sap about three times the volume of the entire root system. Sugar cane stools have been observed to exude 100 ml. or more of sap per day for a week or longer after detopping (263), and corn plants in the milk stage have yielded over 500 ml. of sap in three days and over 1,700 ml. in 15 days (164). Four squash plants grown in nutrient solution and transferred to tap water yielded over 550 ml. of sap in 24 hours, though the total fresh weight of their root systems was only 450 grams. The

volume of sap exuding from these root systems in 24 hours was thus greater than the total volume of the root system and amounted to more than five times the estimated volume of the lumina of the xylem vessels (54).

Composition and osmotic pressure of exudate. The liquid exuding from cut stems and hydathodes is not pure water, but contains varying proportions of carbohydrates, nitrogenous materials, organic acids and mineral salts. The sap of the sugar maple contains an average of over 3% sucrose and small quantities of proteins, minerals, especially calcium and potassium salts, and organic acids, especially malic acid (128). The principal carbohydrate in birch is said to be glucose (41), and an analysis of grape sap gave a total of 1.56% solids, 0.56% being ash, 0.56% organic acids, 0.33% reducing sugar, and small quantities of organic and inorganic nitrogen (204). Weller (263) has published analyses of sap from Hawaiian sugar cane, Pierre and Pohlman (200) summarized the analyses of several investigators and Anderssen (3) cites a number of investigations of woody species. Practically all these data were based on sap exuding from cut stems.

Since woody species usually exude sap only for a limited period in the spring, attempts have been made to obtain samples of xylem sap at other seasons by centrifuging (7) and by the use of liquid or gas pressure (3, 12). Sap centrifuged from the xylem of several woody species in Ireland had osmotic pressures of about 0.5 to 1.5 atmospheres (67, 68). In general, the concentration of solutes was highest in early spring, was very low during summer and early autumn, increased slowly during the winter and suddenly reached its peak again in the spring. Similar changes in concentration were observed in sap extracted by air pressure from apricot and pea stems in California, though the concentration level was lower (3).

The possibility of analyzing the sap exuding from the stumps of detopped herbaceous plants and using the results as a guide in determining the adequacy of the supply of minerals has been discussed by several investigators. Xylem sap obtained from corn has a composition much nearer the composition of the soil solution than does sap expressed from the tissues (164). It has been found to give a good measure of phosphate supply and

probably is a good indicator of the available supply of nitrogen and potassium (200). Considerable discrepancies existed between the composition of the exudate from sugar cane and the composition of the soil extract (263). One investigator (153) suggested that the best method of obtaining samples of soil solution is to displace it through plant root systems by air pressure. It has been suggested that the concentration of certain elements, especially nitrate nitrogen, in the conducting system may affect the growth of microorganisms and the severity of certain diseases of the vascular system (232).

Magnitude of root pressure. The first measurements of root pressure known to the writer are those of Hales (92) who observed pressures of more than one atmosphere in grape. Pressures of 2.6 atmospheres have been reported for birch in New England (41, 181), and according to Huber (117) a Japanese found *Cornus controversa* to develop a maximum pressure of over 1.9 atmospheres. The maximum pressure developed by sugar cane was 1.9 atmospheres, and the most drought-resistant varieties maintained the highest pressures during periods of decreasing soil moisture (263). It was reported that excised tomato roots growing in culture solutions exude sap with a pressure in excess of six atmospheres and probably equal to at least ten atmospheres (265). This probably is the highest true root pressure ever recorded and is the more remarkable because it was produced by isolated roots only a few centimeters in length. Boehm (18) and Figdor (78) observed pressures of over eight atmospheres in stems of woody species, but Molisch (188) claims these pressures were of local origin, and MacDougal (170) says the four-atmosphere pressure observed by him in Monterey pine was also of local origin and not caused by root pressure.

Sap exuding from wheat seedlings is reported to have an osmotic pressure of about 1.3 atmospheres (166), and a Russian (227) by indirect means calculated the exudation pressures of various herbaceous species to range from 0.5 to 1.5 atmospheres. Magnitude of root pressure is affected by the environment of the roots, none being observed in cold or dry soil, or in a poorly aerated medium. The root pressure of tomato plants grown in full strength Hoagland solution was higher than that of plants grown in half strength solution, the maximum root pressure observed being about two atmospheres (250).

Periodicity of exudation. Ever since the early studies of Hofmeister (112) investigators have observed fluctuations in magnitude of root pressure and volume of exudate which seemed to occur independently of variations in the environment (87, 108, 218, 239, 265, 268). Grossenbacher (88) showed that in sunflower there is a well defined periodicity with a maximum and a minimum each 24 hours, the maximum value coming during the day, the minimum at night. If plants are grown in darkness with artificial light supplied only at night the cycle is shifted so that the maximum occurs during the artificial day. The 24-hour cycle also exists in plants when grown with continuous artificial light, but the time of occurrence is determined by the time at which the plants are detopped. No satisfactory explanation of the causes for this definite periodicity can be offered (238). Considerable variations in the volumes of water absorbed and exuded by excised onion roots and root segments have also been observed by Rosene (222) who also found the ratio of absorption and exudation was quite variable at first, but usually reached unity in 24 hours.

Tissues involved. It is ordinarily assumed that root pressure is developed in the water-conducting elements of the xylem and that the exuding liquid comes from the open ends of these elements. This view was challenged by two English investigators (123) who claimed that exudation occurs from the phloem of *Acer pseudoplatanus* L. Exudation from the phloem has been observed in some species (54, 61, 65, 118, 193), but it is usually most abundant in woody species in late summer (191), and the quantity of sap obtained is very small compared to that obtained from the xylem in the spring. Other European workers who reinvestigated this problem reported sap to exude only from the xylem of birch and *Sanchezia nobilis* Hook (116, 121). The writer found clear evidence that in the spring exudation occurs only from the xylem of wounded *Acer rubrum* L., *Betula nigra* L. and *Carpinus caroliniana* Walt., and exudation from the xylem has been observed in numerous herbaceous species which have been carefully observed (54, 143). It appears that the English investigators misinterpreted their results because they failed to take into account the marked diurnal variations in sap flow caused by the alternation of positive and negative pressures in the water-conducting system of woody plants.

Guttation. By far the most common and conspicuous manifestation of root pressure is the exudation of drops of liquid from the edges and tips of leaves, a process termed "guttation" by Burgerstein (32).   Guttation occurs through hydathodes which consist of stomate-like pores in the epidermis below which are large chambers surrounded by masses of thin-walled parenchyma cells.   The xylem of a small vein terminates beneath each hydathode, and presumably root pressure forces water out of the xylem, flooding the intercellular spaces and causing an overflow through the pores to the exterior of the leaf. Guttation also occurs through ordinary stomates of some grasses and legumes, and secretion independent of root pressure occurs from various specialized epidermal cells and hairs (91, 156). Haberlandt (91) distinguished between epithem hydathodes from which water is forced by root pressure and active hydathodes from which water is secreted by forces developed in the cells themselves. It seems preferable to term the active hydathodes "glands" and the outflow of liquid "secretion", since the force responsible for it is entirely different from that causing guttation from epithem hydathodes. While most plants exude only a few drops of water during an entire night, species of *Colocasia* have been reported to exude from 10 to 100 ml. in a night. Although *Colocasia antiquorum* is said to exude almost pure water (69), the guttation liquid usually contains low concentrations of sugar and salts (58, 60, 270), and that of certain species contains so much salt that the leaves become incrusted. Instances have been reported of injury to leaves, in the nature of tipburn, by concentration of solutes caused by evaporation of the guttation water and also by solution of spray materials in the water which is sometimes reabsorbed into the leaves (59, 122). It is also claimed that conditions favorable to guttation often result in water-soaking of the leaves, facilitating infection by pathogenic organisms which otherwise would have difficulty in gaining entrance (125).

More recent investigations have substantiated the long-held opinion that guttation is caused chiefly by hydrostatic pressure originating in the roots (190, 271). Montford (190) regarded guttation as a sensitive indicator of the availability of water to plants and used it in his studies of the water relations of bog plants. Gäumann (84) also regarded it as a valuable indicator

of the condition of the roots. Leonard (154) has recently used change in root pressure as a measure of injury to cotton root systems by cultivation.

Guttation is most common in plants growing in moist warm soil with their tops surrounded by humid air. It occurs only when absorption is sufficiently in excess of transpiration to cause development of a positive pressure in the xylem elements. Conditions whch hinder water intake, such as cold or dry soil, a high concentration of solutes and the presence of toxic substances, also conditions which favor high transpiration, reduce or prevent guttation. These factors will be discussed in more detail in connection with factors affecting water intake.

An interesting example of exudation of water from uninjured woody stems of red maple, analogous to guttation, has been reported (82). In late February sap was observed exuding through the bark of stump sprouts at a height of six to eight feet. It probably escaped through lenticels. Escape of sap from the leaf scars of deciduous species has been observed during warm humid autumns in Louisiana (208).

The root pressure mechanism. No phase of botany has caused more discussion and controversy than the causes of root pressure and the resultant guttation and exudation from woody stems. As Heyl (108) stated in his extensive review, this is partly because of lack of agreement concerning what is to be included under root pressure. Such diverse phenomena as secretion from nectaries and from digestive glands of insectivorous plants, guttation, flow of sap from wounded agave, palm and sugar maple plants and from detopped root systems have all been included by some writers. It is highly doubtful that all these phenomena have the same cause, and it is, therefore, not surprising that different investigators have come to different conclusions. As previously indicated, this discussion is chiefly concerned with pressures which originate in the root system and with the direct results of these pressures on other parts of the plant.

The principal explanations of root pressure which have been offered may be placed in two groups, those which are based on some sort of secretory activity by the root cells and those which assume that the roots behave essentially as osmometers.

a. Vital or secretion theories of root pressure. In accord with

the vitalistic philosophy of the times, most early explanations of root pressure assumed some sort of secretory activity of the root cells. One of the earlier definite theories was that of DeCandolle (63) who believed the root tips, which he termed "spongioles," were contractile organs which sucked in water and forced it into the xylem. His theory somewhat resembles that of Bose (20) which received wide publicity a few years ago. Bose claimed that "the absorbing root-cells are continuously stimulated by mechanical friction against the soil, giving rise to peristaltic waves of pulsation along the active propulsive layer of the inner cortex". Hofmeister (112) first reported the occurrence of root pressure in herbaceous plants and first observed periodicity in amount of exudate and pressure. He believed that absorption of water by the parenchyma cells of the roots and the resulting turgor pressure caused a unilateral movement of water through these cells and its expulsion from the inner cells into the intercellular spaces and the xylem vessels. Wieler (268), Pfeffer (199), Lepeschkin (155) and others believed that root pressure occurs because the root cells are more permeable on the inner than on the outer side and therefore absorb water from the exterior and lose it to the xylem on the interior of the root. It was suggested by Ursprung (249) that a difference exists in the suction tension (diffusion pressure deficit of Meyer, 183) on the inner and outer sides of the endodermis which is capable of causing the movement of water across the endodermis into the xylem. Several investigators have suggested the importance of differences in electrical potential between the inside and outside of the root. If the tissues in the stele have a lower potential than the tissue near the epidermis, i.e., are negative to the external tissue, then water might move inward by electroosmosis. Stern (241) caused water to move through segments of willow twigs by artificially producing a difference in potential between the two ends. Differences in electrical charge on the inside and outside of the endodermis have been reported, based on staining techniques (9, 130), and Lund (165) found differences in potential across the cortex of roots which he believed might cause the inward movement of water and root pressure. After extensive review of the literature and experimentation Heyl (108) concluded that root pressure is not a simple osmotic process but is probably an electro-osmotic phe-

nomenon in which a potential difference is maintained by means
of energy released by respiration of the root cells. It has also
been claimed that there is a good correlation between rate of
respiration and rate of water absorption by corn roots (105), but
another investigator has failed to observe such a correlation (163).

Renewed interest in the possible role of secretion has been
aroused by claims that the cytoplasm sometimes "secretes" water
into the vacuoles (13, 167, 178). It also has been reported that
the intake of water by detopped root systems and excised roots
can be partly inhibited by KCN (225, 250). Exposure of roots
to KCN not only inhibits water intake but also decreases oxygen
consumption, suggesting that water intake and the resulting root
pressure are dependent on energy supplied by respiration in the
root cells (225). Since inhibition is only partial, Van Overbeek
(250) concluded that root pressure is caused by two forces, a
simple osmotic process not affected by cyanide and a secretory
process inhibited by it. Rosene (225), however, suggested that
failure to completely inhibit the root pressure mechanism may be
because KCN does not completely inhibit respiration. More re-
cent work (57) does not support the view that water is secreted
into the vacuole, and it is possible that the inhibiting effect of
KCN on root pressure is indirect and caused by reduction of
solute accumulation in the roots rather than by inhibition of any
secretory mechanism.

b. *Osmotic theories of root pressure.* Our present day views
on plant water relations are strongly influenced by the work of
Dutrochet (73) who developed the theory of osmosis and applied
it to numerous plant processes. He was somewhat vague, however,
concerning the details of the osmotic explanation of root pressure,
and during the latter part of the 19th and early part of the 20th
century most botanists preferred some sort of explanation based
on secretion into the xylem. This was largely because of inability
to understand how water could move from the parenchyma cells
surrounding the xylem into the vessels. It was assumed that
water moves only along gradients of increasing osmotic pressure,
and it was therefore difficult to explain how it could move from
the living cells surrounding the xylem elements, which have an
osmotic pressure of several atmospheres, into the xylem elements
whose contents usually have an osmotic pressure of less than two

atmospheres. The first really workable osmotic theory seems to be that proposed by Atkins (7) who stated "that the influx of water from the ground to the elements of the wood of the roots takes place across the cortical cells of the root. For though they have a much higher osmotic pressure than have the tracheae they function merely as a complex, semipermeable membrane as they are already fully distended". It is now generally agreed that movement of water in plant tissue occurs along gradients of diffusion pressure deficit (hereinafter to be termed D.P.D., following Meyer, 183) which may be largely independent of the relative osmotic pressures of the cells (17, 202, 246). Another problem still existed, however, with respect to the maintenance of a sufficiently high concentration of solutes in the xylem to cause a gradient of diffusion pressure deficit across the cortex from soil to xylem. Atkins (7) suggested that the adjoining parenchyma cells secrete sugar into the xylem vessels, thereby maintaining the necessary concentration of solutes. Priestley (202) believed that these solutes might be supplied from the contents of cells which are differentiating into xylem vessels.

Recently an interdependence between root pressure and ion absorption has been proposed by Crafts and Broyer (55). Since ion absorption and retention are related to rate of metabolism and that in turn to oxygen supply, the tissue in the interior of the root is less able to retain a high concentration of ions than the better aerated tissue at the periphery. A gradient therefore should exist across the root tissues normally accompanied by loss of solutes from the innermost parenchyma cells into the xylem vessels, thus maintaining the concentration gradient needed for osmotic movement of water. The mechanism recently proposed by Lundegardh (166) seems to be similar to that of Crafts and Broyer. A weakness of this theory is that it does not explain the presence of carbohydrates in the xylem sap. Hoagland (110) also emphasizes the relation between absorption of salts and occurrence of root pressure. Root pressure and guttation are exhibited only when root systems are in well aerated dilute salt solutions maintained at favorable temperatures. Guttation soon ceases from plants with roots immersed in distilled water or in unaerated solutions, conditions which also inhibit absorption of salt.

Some writers have emphasized the importance of the endo-

dermis in providing a sheath around the stele through which water can move only by passing through the protoplasm of the cells, but cannot pass through the suberized radial walls (55, 202). This is supposed to prevent leakage out of the stele under pressure and thus facilitate the development of hydrostatic pressure. Since passage cells with unsuberized walls usually occur opposite the xylem points, the writer considers the role of the endodermis to have been exaggerated.

The chief requisites for osmotic movement of water into the xylem are that the cortical cells be saturated and that the D.P.D. of the liquid in the xylem vessels be higher than that of the solution in contact with the roots. It has been demonstrated that water movement can occur across a multicellular living membrane even when the osmotic pressure of the cell contents of the membrane is much higher than the osmotic pressure of the solution inside the membrane (136). Various determinations of the osmotic pressure of the xylem sap previously cited indicate maximum values of one to two atmospheres which is materially higher than the osmotic pressure of most soil solutions. The highest osmotic pressures in the xylem sap of woody plants have usually been observed in the spring which is the season when root pressure also is usually greatest.

c. *Secretory versus osmotic theories.* Considerable discussion has occurred concerning the relative merits of the two types of explanations of root pressure and associated phenomena. It has been strongly argued that root pressure can be adequately explained as the result of osmosis, caused by a difference between the D.P.D. of the xylem solution and that of the medium surrounding the roots. Exudation is quickly stopped by immersion of roots in dilute solutions, and the rate of exudation of cotton is proportional to the difference between the osmotic pressures of the solution in the xylem and that surrounding the roots (77). Rapid reversal from exudation to absorption and back to exudation can be demonstrated by transfer of root systems from water to dilute sugar solution and back to water (145). Such reversal requires less than a minute, may be repeated numerous times, and is evidence that roots behave as very sensitive osmometers. It may be questioned that a complex secretory mechanism is capable of such rapid reversal.

The reviewer believes that osmotic movement of water occurs and that it is at least partly responsible for root pressure, but a simple osmotic theory is inadequate to explain periodicity in rate and pressure, or the reaction to auxin (238) and KCN (225, 250). Auxin applied to the stems increased the rate of exudation of garden pea and sunflower, and the effect of the auxin was exerted on the mechanism responsible for the diurnal periodicity of sunflower. It was suggested that auxin affects the utilization of food required for continued exudation, but no relationship could be established between exudation and respiration. It has also been suggested that auxin increases the absorption of water by plant tissue because it increases the intake of salts (47), but this view has not been supported by the most recent work (251). Electro-osmosis is not regarded as likely to prove a very important factor because conditions in roots are not regarded as favorable for adequate current flow (250).

On the basis of our present knowledge it seems reasonable to assume that the movement of water into the roots responsible for root pressure is largely dependent on the concentration of salts in the xylem. Accumulation of salts in turn is dependent on the metabolic activity of the living cells of the roots. Therefore factors affecting root metabolism might be expected indirectly to affect the intake of water and the resulting root pressure and exudation. This scheme accounts only for the salts present in the xylem sap and not for the carbohydrates which are often present.

It is likely that our views on this subject will be modified as our knowledge of secretory phenomena in plants and animals increases.

The Passive Absorption Mechanism

It has been at least partly understood ever since the time of Hales that the forces bringing about absorption of water by transpiring plants are probably different from those causing root pressure. Sachs, Pfeffer and Strasburger agreed that transpiration sets in motion the forces which bring about water intake by transpiring plants. The picture was completed by the development of the cohesion theory of the ascent of sap which explained how the forces produced in the top by loss of water in transpiration could be transmitted through the water in the xylem to the

roots and there bring about the intake of water. Dixon (65) suggested that water may be regarded as moving through the transpiring plant along a gradient of decreasing vapor pressure from soil to roots, to leaves, and thence into the air. Renner (212) clearly differentiated between absorption brought about by forces originating in the roots and forces originating in the tops.

Evaporation of water from the mesophyll cells of the leaves produces a D.P.D. gradient which causes water to move into them from the xylem of the leaf veins. This reduces the pressure on the water in the xylem, and if transpiration is removing water more rapidly than it is being absorbed, as it often is, the water in the xylem is placed under tension. Subjecting water to tension lowers its vapor pressure and produces a D.P.D. numerically equal to the tension. Since there is a continuous liquid system extending through the xylem to the roots, this D.P.D. is transmitted to the roots and there produces a D.P.D. gradient along which water moves from the epidermis into the xylem. Some discussion has occurred concerning the relative importance of osmotic and imbibitional forces in absorption. Obviously water moves through both cell walls and vacuoles, and since they are in contact with each other the osmotic and imbibitional forces tend to come into equilibrium with each other (234). By discussing water movement in terms of D.P.D. gradients, argument on this subject can be avoided. If the tension in the xylem sap exceeds the osmotic pressure of the cortical cells of the root, the sap in these cells is also subjected to tension and the difference in D.P.D. exists directly between the soil and the epidermis, rather than between the soil and the xylem. Since the osmotic pressure of the root cells is usually rather low and since tensions of considerable magnitude are supposed to occur in xylem sap, it is probable that the root cells are frequently under tension.

Under these conditions roots act essentially as passive absorbing surfaces through which water is absorbed. That this is essentially true is indicated by the fact that shoots may remain alive for some days after their roots are killed (137) and that water intake by a root system can be greatly increased by attaching a vacuum pump to the stump (128, 136, 213). Various aspects of this problem are discussed in the following papers (141, 157, 235).

Relative Importance of Active and Passive Absorption

Considerable evidence is available concerning the relative importance of active and passive absorption in the water economy of plants. In the first place there are many species, including the gymnosperms, which are not known to exhibit root pressure or other evidences of active absorption. Furthermore, among those species in which root pressure is of regular occurrence it is seldom observed in transpiring plants. Water and dye are commonly absorbed through incisions made into the xylem of transpiring plants, but water rarely exudes until after an appreciable interval, if at all. When the tops are removed from freely transpiring plants, usually no immediate exudation occurs from the stumps, but water is absorbed for a period varying from a few minutes to a few hours (141). Exudation apparently begins only after the root cells have become fully turgid by absorption of water through the stump and from the soil.

Perhaps the most important evidence concerning the relative importance of active and passive absorption is a comparison of the volume of water exuding from a detopped root system with the volume lost in transpiration by the same plant prior to removal of the top. In one series of experiments the volume of exudation from stumps of potted coleus, hibiscus, balsam, sunflower and tomato plants was only 1% to 5% of the volume of water lost by the intact plants in transpiration (141). Even when a vacuum pump was attached to the stumps the volume of exudation rarely equalled the volume lost in transpiration by intact plants (141, 213, 254). It has been suggested (132, 218) that the tension produced by transpiration stimulates the root cells and increases active absorption, but no evidence of root pressure has been found in the roots of transpiring plants (19). In fact removal of the root system is often followed by a considerable temporary increase in the rate of absorption by transpiring shoots (140).

Renner (211) long ago presented evidence that the forces developed by transpiring shoots are much more important in water intake than the forces developed in the roots. Considerable evidence is available indicating that root systems are unable to absorb water from as concentrated solutions as are intact plants. The absorbing power of a detopped root system is relatively low.

Maximov (179, *53-55*) cites Russian work (227) in which it was found that exudation from the stumps of various species of plants was generally stopped by solutions with an osmotic pressure of 0.5 to 1.5 atmospheres. Exudation from the stumps of sunflower, castor bean and bean was stopped by sucrose solutions with osmotic pressures of 1.6 to 4.2 atmospheres (213). While intact bean plants could absorb from solutions with osmotic pressures up to 14.6 atmospheres, their root systems could absorb from solutions with osmotic pressures no higher than 1.9 atmospheres (245). The effects of dilute solutions in hindering or even reversing the movement of water through root systems has been observed by several other investigators (77, 136, 141, 213, 221). Detopped root systems do not exhibit exudation in soil drier than about half way between the moisture equivalent and the permanent wilting percentage, but exhibit exudation as soon as the soil is wetted (145). Evidently about half the soil moisture available to an intact plant is unavailable to the root system alone. There is also both direct and indirect evidence that the active absorption mechanism often fails to function in saturated soil.

Renner (213) estimated that the roots of transpiring sunflowers develop absorbing forces of 4 to 11 atmospheres, and another worker (132) estimated that a force of 20 to 73 atmospheres must be developed to bring about intake of the volume of water required by a transpiring sunflower. These estimates are probably too high because it has since been learned that as the tension on the water in the xylem increases the area of root surface through which water intake occurs also increases (28). Increasing the area through which absorption occurs materially decreases the force required to absorb a given volume in a unit of time. Warne (254) estimated the tension in the xylem elements to vary from 1.2 atmospheres in *Pelargonium* to 13.1 atmospheres in *Erica*. In general, when transpiration was high the greatest tensions were found in xerophytes and the lowest in mesophytes and succulents. As would be expected, the tensions were reduced or disappeared when the plants were well supplied with water and transpiration was stopped. Since the D.P.D. of soil at the permanent wilting percentage is in the neighborhood of 15 atmospheres (214), it is clear that all transpiring plants must be capable of absorbing water against a deficit at least this great. The root system alone,

however, can absorb only against a D.P.D. of one or two atmospheres.

One reason often given for emphasizing the importance of the metabolic activity of root cells in water intake is that absorption is materially reduced by low temperature and by poor aeration, both of which are known to reduce the rate of respiration. It appears possible, however, that the physical effects of low temperature and poor aeration are sufficient by themselves to explain decreased absorption by transpiring plants (142, 144). This phase of the problem will be discussed with factors affecting absorption.

It is concluded that active absorption, as evidenced by exudation and root pressure, is wholly inadequate to supply the water requirements of transpiring plants. It is probable that active absorption does not even supplement passive absorption in rapidly transpiring plants because none of the suggested mechanisms for active absorption would operate unless the cells of the roots were turgid. Water absorption by transpiring plants apparently is caused by forces set in motion by the loss of water in transpiration. Removal of water by transpiration decreases the pressure or causes tension on the xylem sap, producing a gradient of decreasing pressure and increasing D.P.D. along which water moves from the epidermis to the xylem of the roots. The roots act as passive absorbing surfaces in connection with this mechanism, and it may be said that water is absorbed through the roots of transpiring plants rather than pumped into the plant by the roots.

FACTORS AFFECTING ABSORPTION

The factors affecting the rate and amount of absorption may be grouped as plant factors, such as rate of transpiration and extent of the root system, and environmental factors, such as soil moisture, temperature, aeration and concentration of the soil solution.

Plant Factors

Rate of transpiration. If transpiration produces the forces chiefly responsible for the intake of water we would expect the rate of absorption to be closely related to the rate of water loss. A number of studies made at various times and under a variety

of conditions have in general shown a close correlation between the rates of these two processes (150, 159, 190, 252). The writer (139) made simultaneous measurements of absorption and transpiration of loblolly pine, green ash, yellow poplar, black willow, sunflower and opuntia. Sunflower was grown in both soil and water culture, willow in water culture and the other species in soil. In all instances changes in rate of water loss were soon followed by similar changes in rate of water intake. The fact that changes in transpiration precede changes in rate of absorption indicates the close dependence of absorption on transpiration.

Root system.   a. Absorbing zone.   The region of maximum water intake by a root is determined by its anatomical structure. This changes with age in such a manner that the principal absorbing zone is generally supposed to be restricted to the youngest region, near the root tip. Since the anatomy of roots is adequately described in various texts (74, 99, 184), it will not be discussed in this paper.

Several studies have been made of the relative volumes of water absorbed by various regions of the root. Coupin (51, 52) believed that absorption occurs chiefly through the root tips, but it is generally agreed that little water is absorbed through the root cap and meristematic region (29, 113, 203, 237). The exact location of the zone of most rapid absorption varies with the length and age of the root (220), with time (222) and with the tension developed by the transpiring shoot (28). Rosene (220), working with onion roots, found that maximum absorption occurred at the base of roots less than 50 mm. long, but in roots over 70 mm. long maximum absorption occurred 40 to 60 mm. from the apex and decreased toward tip and base. Removal of part of the roots increased the rate of absorption by the remaining roots. Considerable fluctuations in rate of absorption in adjacent areas of onion roots were also observed (222). Absorption by corn roots is said to increase to a maximum about 10 cm. back of the root tip and then usually to decrease proximally in roots more than 10 cm. long (101). It has been observed that the absorbing zone of broad bean is a few centimeters back of the tip with absorption decreasing in each direction from this zone. When the shoot is transpiring rapidly the absorbing zone is extended toward the shoot, including the entire unbranched region (28, 29). It was

suggested that this results from changes in permeability produced by increased tension in the xylem, but it seems more probable that the high tension in the xylem during periods of rapid transpiration causes water intake through tissue which has too high a resistance to water movement to permit entrance at lower tensions.

It has long been assumed that root hairs materially increase the absorption of water by increasing the absorbing surface, but their importance has been questioned by a few workers (51, 52, 113). The zone of maximum absorption as reported by most workers corresponds with the root hair zone, or at least the zone where root hairs would be present if they had developed. Rosene (224) made direct measurements showing that water intake occurs through root hairs of radish with about the same velocity as through the hairless epidermal cells of onion roots. Root hairs have been estimated to increase the absorbing surface of the roots bearing them from five to 20 times (184). The total surface of the root hairs on a four-month-old winter rye plant was 1.6 times the total root surface exclusive of root hairs (64). Root hairs ordinarily live only a few days, but persistent root hairs have been observed on Valencia orange seedlings (103), *Cercis, Gleditsia Gymnocladus* (173) and Venus fly trap (236), and root hairs three years old were reported on certain composites (264). It may be questioned that these persistent root hairs function in absorption, as their walls often become thickened and even suberized and lignified. The absorbing surface of some root systems, especially those of trees, is frequently modified by development of mycorrhizal structures through which much absorption of water and minerals is believed to occur (97, 98).

The epidermis and its root hairs are usually soon destroyed. Sometimes the hypodermis develops a suberized layer which is later followed by another such layer produced from a phellogen originating in the pericycle. In the Valencia orange the hypodermal cells sometimes produce secondary root hairs, lenticels and absorbing areas consisting of groups of thin-walled radially elongated cells (103). Water is presumably absorbed through all of these structures. Usually during secondary growth activity of a phellogen developed from the pericycle encloses the root in a layer of cork and thereby destroys the cortex. The presence of

such a sheath of suberized tissue would be expected to prevent the entrance of water and solutes, but this does not seem to be true. Since at certain seasons, as when the soil is cold or dry, few or no growing root tips can be found on woody species it has been inferred that absorption must occur through the older suberized portions of such roots (39, 56, 194). No direct experimental evidence was presented until recently when it was demonstrated by a potometer study that water can be absorbed through the suberized portions of roots of sour orange (101). The writer (147) recently made a number of measurements of water absorption through attached suberized roots of pine and hardwoods varying from three to 20 mm. in diameter and found measureable absorption to occur through all sizes of roots. It has also been demonstrated that water can be absorbed through dead root systems so long as their conducting tissues remain unplugged (137).

   b. *Extent of root systems.* Since the principal zone of absorption is often near the root tip the number of tips is an important factor in absorption. Furthermore, the continual extension of these root tips through the soil is frequently essential to absorption of adequate quantities of water. This is because in soils with a moisture content below field capacity capillary movement of water toward roots is very slow. The moisture content of the soil in contact with an absorbing root therefore tends to be reduced to the permanent wilting percentage, and unless it is rewetted new supplies of moisture become available only as the roots penetrate into new areas of soil. Contact between roots and soil particles is also believed to facilitate the absorption of minerals (124). Roots elongate fairly rapidly, a rate of 0.5 in. per day being common in grasses, and corn roots are reported to grow downward at the rate of 2 to 2.5 in. per day for three or four weeks (255). Roots of loblolly and shortleaf pine were observed to grow an average of about 0.1 in. per day under favorable conditions (210).

   The literature dealing with the extent and development of root systems is too extensive to be reviewed or even cited adequately in this review. The outstanding investigations are by Weaver and his co-workers, and the reader is referred to their publications for extensive bibliographies on roots and root systems (255, 256, 257, 260). Miller (186) has also summarized much information

concerning factors affecting the development of root systems. A few papers will be discussed. One of the earlier measurements of the extent of root systems was by Clark (42). He found a squash grown in a greenhouse bench to have 84,000 feet (15.9 miles) of roots of which he estimated that 50,000 feet had been added at the rate of 1,000 feet per day. The number and extent of roots is especially great on grasses. A wheat plant grown without competition was found to have 44 miles of roots, a rye plant 49 miles, and a wild oats plant 54 miles (196). Winter rye plants grown four months in boxes of soil in the greenhouse were estimated to have over 13 million roots with a total length of over 387 miles and a surface of 2,554 square feet. These roots bore over 14 billion root hairs with a total length of 6,603 miles and a surface of 4,321 square feet (64). There was an average daily addition of 3.1 miles of roots to each plant. It has been estimated that this rate of root extension would make available 1.6 liters of water daily in a sandy loam at field capacity and 2.9 liters in a heavy clay (149). The root surface of these plants was about 130 times the shoot surface.

c. *Factors affecting development of root systems.* The general physical and physiological characteristics of root systems, like those of other plant organs, depend primarily on their heredity. Nevertheless, wide variations in development of root systems within the limits of their hereditary potentialities are brought about by variations in certain environmental factors. Among these are soil moisture and aeration which are interrelated and in turn are affected by soil texture and structure, soil fertility, soil temperature and competition with other root systems.

Root competition between plants of the same or of different species usually reduces the extent of the competing root systems. Wheat and barley develop root systems nearly 100 times larger when grown without competition than when grown in drill rows six inches apart (196). Planting corn between rows of young apple trees greatly reduced the extent of the root system of the apples, both laterally and vertically (273). Competition with grass reduces the amount and extent of root systems of forest tree seedlings (261), and the vigor and size of root systems of loblolly pine seedlings are reduced by competition with forest vegetation (45). Such competition is quite complex, including

the effect of shading on the processes occurring in the shoots and competition for water and minerals by the roots.

Moisture content of the soil is perhaps the most important environmental factor affecting root development because either too much or too little soil moisture hinders development of roots. It has been claimed that roots are able to grow into dry soil, possibly by transferring water from roots growing in moist areas (26, 27, 231). Other investigators, however, have found roots unable to penetrate more than a few millimeters into dry soil from adjacent moist soil (62, 106, 162), and it seems unlikely that they make appreciable growth into soil at or below the permanent wilting percentage. In the Great Plains region, for example, root penetration is definitely limited by the depth to which water penetrates (258). Hydrotropism is apparently less important in roots than once supposed, being completely absent from some species and weak in others (162).

Everyone is familiar with the yellowing of leaves, reduction in growth and eventual death of crop plants resulting from prolonged saturation of the soil with water. That this damage is caused by injury to the roots from lack of oxygen and possibly by accumulation of carbon dioxide rather than by the direct effects of water is indicated by the satisfactory growth of such species in well aerated water cultures. It is said that saturated soils containing organic matter also usually develop toxic concentrations of ferrous iron, sulfides and manganese (216). In general, the best developed root systems occur in soils with a moisture content which is usually below field capacity but not as low as the permanent wilting percentage. Within certain limits, therefore, root growth is inversely proportional to the moisture content of the soil (71, 114).

Respiration of roots and soil organisms tends to decrease the oxygen content and to increase the carbon dioxide content of the soil atmosphere and of the water films in equilibrium with it. The activity of soil organisms in this respect is affected by temperature, moisture and the supply of nitrogen and carbohydrates. By adding starch and ammonium sulfate to soil such an increase in soil fungi was caused that all free oxygen disappeared and wheat plants growing in it were killed (129). The concentration of gases in the soil atmosphere tends to become equalized

with that of the above-ground air chiefly by diffusion. Aeration is related to soil texture and structure because gas exchange ordinarily occurs through the larger or non-capillary pores which are free of water at moisture contents lower than the field capacity. Aeration is better in coarse-textured sandy soils than in fine-textured clays. This explains the fact that while root systems penetrate five to 35 feet into the light well drained soils of Nebraska and surrounding States, most roots are restricted to the top two or three feet in the East where heavy clay subsoils hinder drainage and aeration. Roots developed during periods of low soil moisture sometimes die after rains or irrigation raise the moisture content too high for good aeration (24, 118).

The earlier work on aeration and root growth has been summarized by Clements (43), and various aspects of the problem are discussed in the following papers which also give numerous references (22, 36, 160, 186, 257, 259). According to Cannon (36), many roots will make slight growth at 0.5% to 2.0% of oxygen, but 8% to 10% is usually necessary for good growth. He found that a higher oxygen concentration is necessary for optimum growth at high soil temperatures than at low temperatures. Apple tree roots can survive for some time at 55° F. to 70° F. in soils containing 0.1% to 3% oxygen (23), but growth was so seriously checked below 10% of oxygen that top growth was noticeably reduced (21). In New York there is said to be a relatively short period of time when enough oxygen for good root growth occurs in clay soils below three feet (24). Flooding the soil around the roots of young apple trees greatly reduced transpiration and photosynthesis, but increased respiration of the tops (40, 229).

Most species require good aeration for proper development and functioning of their root systems, but certain species characteristic of bogs and swamps can survive for long periods in saturated soil. Apparently the root systems of such species can survive with little or no oxygen and probably are able to carry on anaerobic respiration without suffering injury (151, 158). It also seems probable that considerable oxygen diffuses down from the aerial parts to the submerged roots and rhizomes of some species (50, 86, 152). Part of this oxygen probably is released by photosynthesis in the shoot (37, 152). It is well known that most bog

plants have shallow root systems, and some are so rooted on hummocks of sphagnum that their roots are fairly well aerated (53). Trees on poorly drained uplands also sometimes have very shallow root systems (25).

While the roots of many plants will grow during the entire year, growth is usually much slower during winter. Apple roots have been observed to make some growth at depths of 3.5 to 4.5 feet in soil near freezing while the upper two feet were frozen and subzero air temperatures occurred (46). More roots and a higher root-shoot ratio were obtained in white pine seedlings in soil at 88° F. than at any lower temperature (1). Several investigators have observed that while the root growth of trees and other perennials is reduced in winter it does not completely cease unless the soil is frozen (35, 93, 172, 210, 217, 248). Under some conditions temperatures near the surface of the soil may be high enough to injure roots or the lower part of the stems of tree seedlings (11, 134, 197, 233). Very few roots occur in the upper foot of soil in certain California orchards because of the high soil temperatures during summer (205). Some species of grasses show a definite periodicity in root growth which may be in part related to soil temperature and flowering (244).

Roots tend to branch more profusely in very fertile soil than in soil low in available minerals (257). Their development is also generally believed to be affected by soil acidity, though recent work indicates that good plant growth is possible in nutrient solutions over a wide pH range if care is taken to insure the availability of all necessary minerals (6). In arid regions accumulation of salts in toxic concentrations often injures roots and prevents establishment of plants (76, 100, 175, 176).

Since roots are dependent on their shoots for a supply of carbohydrates those factors which affect the amount of photosynthesis also affect root growth. Thus heavy grazing or frequent clipping is well known to reduce the size of the root systems (16, 95, 195, 215). It has also been demonstrated that shading reduces growth and the ratio of roots to shoots in tree seedlings (15, 34, 83, 184, 198).

Environmental Factors

Readily available moisture content of the soil. Since the terminology and the relation of soil moisture to plant growth has

been considered at length in an earlier review (148), it will be discussed but briefly in this paper. Only that portion of the soil moisture which lies above the permanent wilting percentage is available for plant growth. Permanent wilting of plants may occur at less than 1% of water on a dry weight basis in coarse sand, and at 25% or higher in clay. The moisture content of a soil, therefore, can be intelligently interpreted in respect to plant growth only by considering its relation to the permanent wilting percentage and the field capacity of that soil. Some investigators claim that soil moisture is equally available to plants over the range from field capacity to permanent wilting percentage, but there is considerable evidence that the availability of water decreases with decreasing soil moisture from at least half way down the range from field capacity to permanent wilting percentage (145, 148). Some data are available indicating that transpiration decreases with soil moisture contents both above and below the field capacity (184, 266), and this is also true of exudation from detopped root systems. A soil moisture content above the field capacity doubtless depresses absorption by hindering proper aeration of the roots. Decreasing soil moisture content decreases absorption because as the moisture content decreases and the films of water surrounding the soil particles become thinner they are held more firmly. The diffusion pressure deficit of the soil moisture is only about one-half to one-third of an atmosphere at the field capacity, but it is about 15 atmospheres at the permanent wilting percentage.

As the steepness of the diffusion pressure deficit gradient from soil to roots decreases, water movement slows down until finally absorption lags so far behind water loss that wilting occurs. Absorption does not completely cease, however, until the plant dies of desiccation. Movement of water in soils below their field capacity is so slow that it becomes available to plants only if the roots actually come into contact with it. Puri (207) suggested that it is doubtful that the permanent wilting percentage is determined by the decreased D.P.D. gradient from soil to roots. Rather, he believes, it is determined by the amount of moisture held in capillaries too small to be penetrated by root hairs. Thus in fine-textured clay soils with numerous small capillaries more water is unavailable than in sandy soils with few very small

pores.   As the soil moisture content decreases and absorption
becomes slower, growth is checked, and decreased elongation of
root tips probably further decreases absorption.

There is some evidence that soil moisture near a plant is more
available than that at a distance, even though the latter is in soil
occupied by the root system.   For example, the moisture in soil
four feet from corn plants was not absorbed until the moisture
content of the soil near the plant was reduced below the perma-
nent wilting percentage (62).   Results obtained with sorghum
under field conditions are indicative of somewhat similar behavior
(49).   Interpretation of the results of such experiments is com-
plicated by the tendency toward a higher concentration of roots
in soil near the plant than at a distance.

Soil aeration.   Inadequate soil aeration with resultant oxygen
deficiency and carbon dioxide accumulation decreases absorption
directly, and also indirectly by reducing root growth.   The earlier
work on aeration in relation to absorption has been discussed
by Clements (43).   The importance of proper aeration of soil
and water cultures as essential to maximum absorption of both
minerals and water has been emphasized in recent papers (5,
110).   It is suggested that, generally, even in the best aerated
soils, growth of species with a high oxygen requirement is prob-
ably limited by lack of oxygen and possibly by accumulation of
carbon dioxide.   Gilbert and Shive (85) concluded that the
oxygen content of water in equilibrium with the air is not high
enough for maximum growth of some crops.   There are consid-
erable differences in oxygen requirements of roots of various
plants, the optimum concentration for tomato being so high that
it was toxic to soybeans (85).   Injury to tomato roots from ex-
cessive aeration at high temperatures has been observed (5), and
other workers have reported injury from excessive aeration (160).

Tomato plants growing in unaerated tanks were observed to
wilt on warm days, but recovered when air was bubbled through
the tank (5), and several other examples of reduction in water
intake by plants with roots in poorly aerated media have been
published (145).   Little information is available, however, con-
cerning the relative importance of oxygen deficiency and accumu-
lation of an excess of carbon dioxide.   In India several species
of trees were quickly injured or killed by development of a heavy

sod over their root systems, and it was concluded that at least part of this injury resulted from an excess of carbon dioxide produced by the grass roots (115). The writer (142) found that saturating the soil or water surrounding the roots of tomatoes and sunflowers with carbon dioxide reduced transpiration one-third to one-half within an hour. Saturating the water around the roots with nitrogen reduced transpiration less than 10%. Exudation from detopped root systems was reduced about two-thirds by carbon dioxide, but only about 10% by nitrogen. Exudation from root systems attached to a vacuum pump was reduced one-third to one-half by carbon dioxide, but only 10% by nitrogen. These results seem to indicate that absorption was reduced more in the first two hours by an excess of carbon dioxide than by a deficiency of oxygen. The large reduction in water intake by roots of transpiring plants and root systems attached to a vacuum line indicated that carbon dioxide decreases the permeability of the protoplasmic membranes of the roots of these species to water. Hoagland and Broyer (111) reported that a high concentration of carbon dioxide in the solution surrounding the roots abruptly decreased absorption of both bromide and water by roots attached to a vacuum pump. Later absorption of bromide and water increased. Apparently carbon dioxide first decreased permeability but later so injured the protoplasm that permeability to water and salts was increased. Bubbling nitrogen through the solution around the roots decreased permeability, but there was no subsequent increase. This presumably is a reaction to oxygen deficiency. Chang and Loomis (38) reported that bubbling CO_2 through the solution surrounding the roots of maize, rice and wheat decreased absorption of water and minerals, but N_2 did not affect absorption. They concluded that CO_2 has specific effects on absorption of water and solutes.

There is some evidence, however, that over periods of more than a few hours, oxygen deficiency may be more important in reducing absorption than is accumulation of carbon dioxide. Plants of coleus, corn, cotton, sunflower, tobacco and tomato were grown (267) in sand-filled containers arranged for aeration with various gas mixtures. The mixtures used were compressed air, 20% CO_2 plus 20% O_2 plus 60% N_2, pure N_2, and 20% CO_2 plus 80% N_2, and the roots were aerated at a rate of two liters

per plant per hour for seven to 14 days. Lack of oxygen decreased transpiration of all species and caused death of many roots of coleus, tobacco and tomato. Aeration with 20% CO_2 in the presence of O_2 did not reduce transpiration of corn, cotton or sunflower, and only slightly reduced that of coleus, tomato and sunflower. An excess of CO_2 in the absence of O_2 reduced transpiration of most species little more than O_2 deficiency alone, but coleus was killed in five days, and transpiration of tomato was decreased more by CO_2 alone than by O_2 deficiency alone or by CO_2 plus O_2. Growth was also reduced more by O_2 deficiency than by an excess of CO_2.

Injury to root systems when poorly aerated presumably largely results from accumulation of toxic products of anaerobic respiration. The lower energy output of anaerobic respiration may also be responsible for decrease in active absorption of water and decreased intake of minerals. Possibly this decrease is partly the result of decreased permeability. It has been suggested that permeability is related to metabolism because maintenance of protoplasmic membranes is dependent on metabolic activity. Apparently permeability, metabolism, salt accumulation, and possibly active absorption of water are closely related (111). Reduction in or cessation of active absorption cannot explain the large reduction in absorption of transpiring plants, however, because active absorption can supply only a small percentage of the water needed by rapidly transpiring plants. The reduced absorption by inadequately aerated roots of transpiring plants must result from decreased permeability of the roots and from decrease in absorbing surface caused by death of rootlets and cessation of root growth.

Soil temperature. It has been known at least since the time of Sachs that low soil temperature reduces the absorption of water by plants and that not all species are affected to the same extent. In general, as might be expected, plants which are native to warm climates and normally grow in warm soil exhibit a greater reduction of water intake when the soil is cooled than do plants which normally grow in cool soils. An extensive comparison of various species was made by Döring (70) whose data indicate that water intake of plants from northern flat moors and high moors was reduced less at low temperatures than was water intake

of plants from warmer and drier soils. Firbas (79) also found that high moor species absorb water freely at low temperatures. Water absorption by Bermuda grass, a native of warm regions, was sufficiently retarded at 10° C. to cause wilting, while bluegrass was unaffected at this temperature (30). The writer (146) found that watermelons and cotton, which are warm season crops, absorbed only 20% as much water at 10° as at 25° C., while Georgia collards, a cool season crop, absorbed 75% as much water at 10° as at 25° C. Loblolly and slash pine, which are southern species, absorbed only 40% as much water at 10° as at 25° C., while the northern species, red and white pine, absorbed 60% as much. Kozlowski (135) found that low soil temperatures reduced transpiration of loblolly pine more than that of white pine. Reduction in water intake at temperatures well above freezing has been reported for a number of species, including citrus fruits (14, 90), cotton (4), cucumbers (230), muskmelons (209), sugar cane (72), sunflowers (44) and willow (206). Serious injury to cucumbers has been reported as a result of watering greenhouse beds with cold water in winter (230), and a chlorosis of greenhouse-grown gardenias was found to occur only in soil cooler than 18° C. (127). Cold soil may be of some ecological significance with respect to plant distribution, and winter injury may sometimes result from desiccation caused by inadequate water absorption from cold soil in sunny weather (185, 226).

It is not surprising that soil temperature often exerts such a marked influence on water intake, since it can be affected in a number of ways by low temperature. Cold soil retards root elongation and thereby reduces root penetration into new soil masses, a matter of importance in soils below their field capacity. The water-supplying power of the soil, as measured by soil point cones, is only one-third to one-half as great at freezing as at 25° C. (138). The viscosity of water is twice as great at 0° as at 25° C., and the vapor pressure is only one-fifth as great. The viscosity of protoplasm is three or four times greater near freezing than at room temperature (262), resulting in greatly increased resistance to water movement through the cell membranes. The writer found active absorption as measured by exudation from detopped sunflower and tomato root systems to be most

rapid at 25° C. It decreased at higher and at lower temperatures, ceasing at about 12° in tomatoes and about 2.5° C. in sunflowers. Wilting of transpiring plants in cold soil cannot be attributed to decrease in active absorption because it normally supplies so little water to the top. It was found, however, that water movement through root systems in soil and in water attached to a vacuum pump was less than one-fifth as great at freezing as at 25° C., indicating that there is a high resistance to water movement through the roots at low temperatures. Water movement through dead roots was about half as great at freezing as at 25° C., indicating that the resistance was decreased but not eliminated by destruction of the protoplasmic membranes. In view of these results it is concluded that the principal cause of decreased water absorption by transpiring plants at low soil temperatures is increased resistance to water movement across the living cells, resulting from the combined effects of decreased permeability of the root membranes and the increased viscosity of the water itself (144).

Concentration and composition of the soil solution. Absorption of water depends primarily on the existence of a diffusion pressure deficit gradient from soil to roots. The plant is potentially capable of developing a D.P.D. at least equal to the osmotic pressure of its cells. This averaged 14.4 atm. for plants from the vicinity of Cold Spring Harbor, N. Y., and much higher for plants of dry habitats (94). Furthermore, as the soil moisture content decreases, the osmotic pressure of the plant tissues increases, thus tending to maintain a gradient from soil to roots (109, 133, 171, 177, 186, 242). The D.P.D. of the soil moisture is the sum of the pressure potential (capillary potential) of the soil moisture and the osmotic pressure of the soil solution.

In most cultivated soils the osmotic pressure of the soil solution is negligible, but sometimes it is so high as to inhibit plant growth. This occasionally occurs in greenhouse soil as a result of excessive applications of fertilizer (48). It is highest in arid regions where rainfall is insufficient to leach away the salts accumulated near the surface by evaporation of water. In the western states best yields are obtained on soils with an osmotic pressure of less than two atmospheres. Good yields of some species are obtained up to an osmotic pressure of four atmos-

pheres, but growth and yield decrease rapidly with increasing concentration. At concentrations higher than 40 atmospheres all species fail to grow (176). Because of the agricultural importance of this problem much work has been done on the effects of salt concentration on the growth of crop plants. Increased salt concentration has been shown to decrease transpiration and water requirement (75, 182), absorption of water (76, 104, 161, 221) and growth of various species (8, 102, 176). Although plants grown in media high in salt are reduced in size and yield, they often have a higher water content than plants grown in media low in salt (102, 182, 253). There are several causes for the decreased absorption of water in the presence of salts besides the increased D.P.D. of the soil solution. Perhaps the direct osmotic effects on absorption are less important than the effects on the extent and efficiency of the root systems. Root growth is inhibited at high salt concentrations (76), and roots are unable to extend into new soil masses. Furthermore, these slow growing roots tend to become suberized to their tips (104) and thus possess less efficient absorbing surfaces than rapidly growing roots.

The osmotic pressure of the soil solution is apparently more important than the type of ions present. Root growth and water uptake of corn and tomato were affected to the same extent in a complete nutrient solution and a chloride solution of the same osmotic pressure (76). Peach trees were found to tolerate slightly more sulfate than chloride, but the difference was small (103a). It was suggested by Meyer (182) that the differences which he observed in retarding effects of various salts on transpiration might largely be caused by differences in osmotic pressure of equimolar solutions rather than by specific ionic effects. Possible differences in effect on permeability of cell membranes cannot entirely be overlooked, however. Solutions of 0.01 M HCl and NaOH were found to retard water absorption by willow (269).

WATER ABSORPTION IN RELATION TO OTHER PROCESSES

Relation of Intake of Solutes to Intake of Water

Most of the early physiologists supposed that absorption of minerals was proportional to the absorption of water, although

very little experimental evidence was presented. Later, various species of plants were grown under conditions favoring high and low rates of transpiration and their salt contents compared (33, 89, 96, 131, 174, 180, 192). The results of these experiments indicate that the volume of salts absorbed is not proportional to the volume of water. Such experiments are not conclusive because plants grown in sun and shade or with high and low humidity are anatomically and physiologically different and cannot properly be compared. In certain recent experiments this objection was eliminated by exposing plants to high and low humidity for only three or four days and comparing salt and water intake (81, 272). In these experiments there was some increase in salt absorption with increased absorption of water, but different ions were not absorbed at the same rate, and the rate of absorption of various ions differed in different species. Hoagland and his colleagues have emphasized the dependence of salt absorption and accumulation by roots on the metabolic activity of the root cells (110, 111). They have shown that excised barley roots can absorb as much salt in a short period of time as similar intact transpiring plants. In other experiments plants supplied with minerals only at night when transpiration was low absorbed as much salt as plants with their roots in nutrient solution only during the day or during the entire 24 hours. These and other experiments indicate that while transpiration may not be without effect (31), the quantity and kind of ions absorbed is usually determined by the metabolic activity of the roots rather than by the volume of water absorbed (110).

Absorption in Relation to Other Processes

The relation between rate of absorption and rate of transpiration is more important than the absolute rate of either process because it determines the internal moisture content of plants and thereby profoundly affects many physiological processes. Although there is relatively little resistance to water movement through the xylem itself (254, 269), there is considerable resistance to movement through the mass of living cells lying between the epidermis and xylem of the roots (140). As a result, on days when transpiration is rapid, absorption lags so far behind transpiration that the moisture content of the plant is ma-

terially reduced and wilting often occurs, even of plants in moist soil or in well aerated liquid cultures. Well defined diurnal variations in moisture content result, especially in the leaves. On a typical day, moisture content decreases during the morning and early afternoon and reaches a minimum in the late afternoon. It then increases during the night, reaching a maximum about midnight or later (139, 240). Acompanying this diurnal variation in water content is a similar variation in the osmotic pressure and D.P.D. of the leaves (107, 242). Considerable redistribution of water may occur between various tissues of a plant as a result of local water deficits. Best known is the removal of water from fruits by transpiring leaves (10, 168). Water is also removed from mature cotton bolls during periods of rapid transpiration, but not from immature bolls (2).

Water deficit affects other internal processes and conditions which profoundly influence the growth, development and even the survival of the plant. Among them are stomatal opening, rates of transpiration, photosynthesis and respiration, the starch-sugar equilibrium, and cell division and enlargement (117, 184, 179).

SUMMARY

Intake of water apparently is brought about by two independent processes differentiated by Renner as the active and passive absorption processes. When soil moisture is abundant and transpiration slow, absorption often exceeds water loss, resulting in the development of positive pressure or "root pressure" in the xylem. This pressure causes guttation and exudation phenomena. Since the absorption mechanism responsible for root pressure is dependent on the presence of active living cells in the roots, it is termed "active absorption". Some workers believe root pressure is caused by secretion of water into the xylem by the surrounding living cells. Others believe it is a relatively simple osmotic phenomenon caused by a difference in concentration of solutes in the xylem elements and in the solution surrounding the roots. Certain similarities between the conditions necessary for salt accumulation and for development of root pressure suggest that they are interrelated to the extent that the occurrence of active absorption and root pressure are at least partly dependent on the accumulation of salt in the xylem and so

indirectly related to metabolic activity and the permeability of the root cells.

During periods of rapid transpiration or when soil moisture is deficient no root pressure occurs. Instead, the water in the xylem is under reduced pressure or even tension. This increases its diffusion pressure deficit and produces a gradient of increasing diffusion pressure deficit and decreasing pressure along which water moves from the external solution into the xylem. Since under these conditions the roots act simply as absorbing organs and water intake appears to be independent of any secretory or osmotic activity of the root cells, this type of water absorption is termed "passive absorption".

Active absorption ordinarily can supply less than 5% of the water required by a rapidly transpiring plant. It does not occur from as dry soil nor from as concentrated solutions as does passive absorption of transpiring plants. Some species never exhibit any root pressure or other evidence of active absorption. It is therefore concluded that the root pressure or active absorption process is of negligible importance in supplying water to plants.

The rate of absorption of water by plants in moist soil is determined primarily by the rate of transpiration. It is affected to a lesser degree by the extent and efficiency of the root system. Important environmental factors affecting absorption of water are the available moisture content of the soil, concentration of the soil solution, soil aeration and soil temperature.

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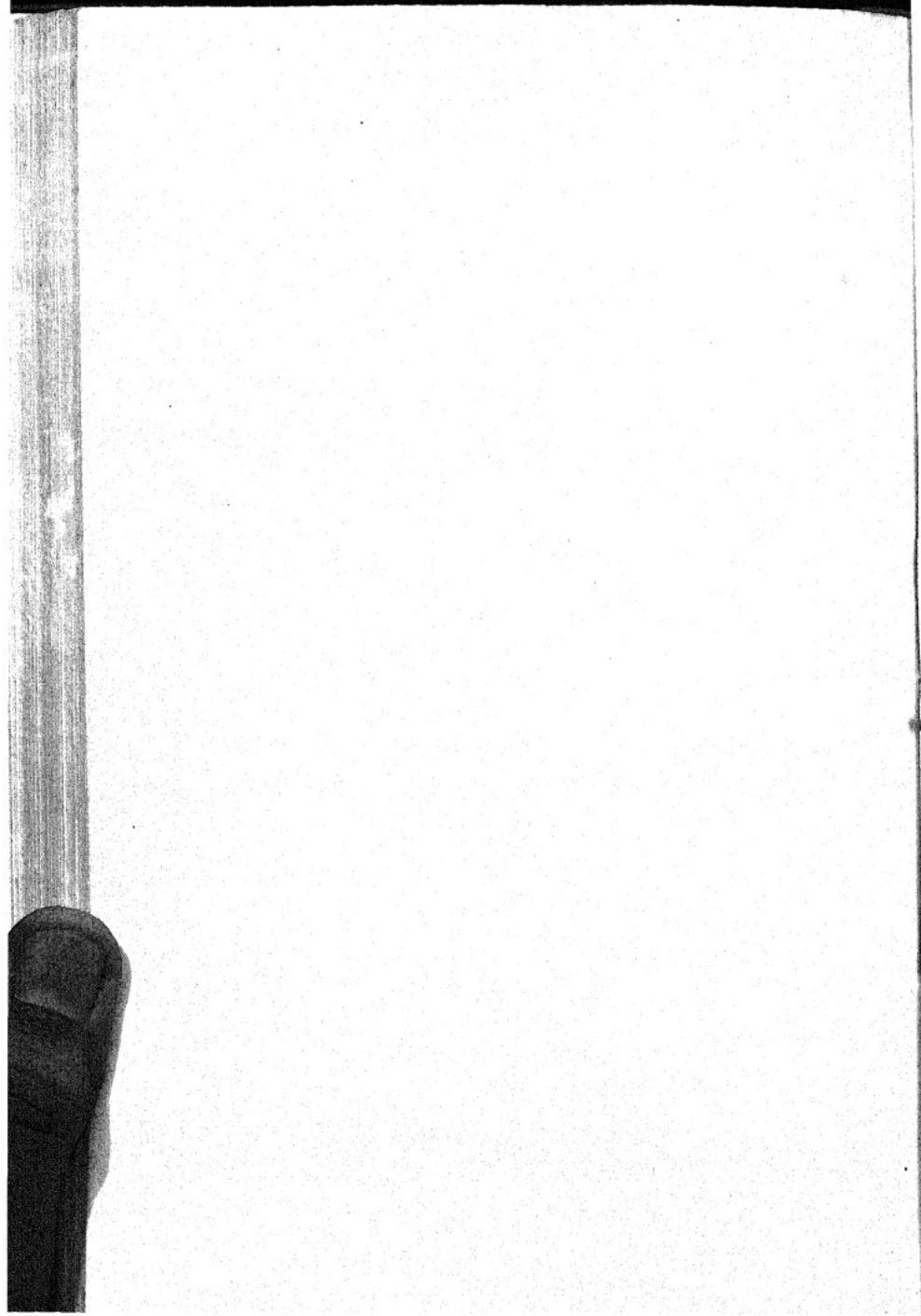
THE BOTANICAL REVIEW

QUANTITATIVE BIOASSAY OF FUNGICIDES
IN THE LABORATORY

JAMES G. HORSFALL

Connecticut Agricultural Experiment Station

INTRODUCTION

Some unkind wag has said that if all the scientists of the
world were placed end to end they would never reach a conclu-
sion. Although scientists resent this statement most bitterly, it
is true enough to get a laugh. Its kernel of truth lies in the fact
that nature is so complex that she presents a different face to
nearly every experimenter. Nature could not present so many
faces if the cameras of the experimenters had wide-angle lenses
or if they were set up in enough places to cover all of nature's
poses.

It is the purpose here to set down and to evaluate critically
the progress that has been made in designing proper equipment
to reach conclusions regarding that little segment of nature known
as fungicidal action. The discussion will be limited primarily to
the laboratory techniques that have been devised for testing
chemicals. The action of heat will be omitted.

A few years ago it was customary to take a dim view of ac-
celerated techniques of assaying fungicides, probably because
results from them unhappily would not check with field results,
or even with themselves. The first criticism was hardly justified
because field results seldom checked themselves. Martin (68)
has well said that "The extent to which the laboratory trial will
be confirmed by field trial will be controlled by the correctness
of the allowances made for the influence of the missing factors
in the interpretation of the results". During the time since Mar-
tin's paper was published, so many "missing factors" have come
under control that we now think that if a compound does not
work in the field when it does work in the laboratory, the field
technique should be questioned before the laboratory technique.

The discussion will be limited primarily also to bioassay techniques because, as yet, chemical techniques have not caught up with bio-techniques. Fungi are much more vocal in expressing their preferences or rather dislikes for different fungicides than known chemical reagents have been. When the chemists will have polished up the techniques for fungicide assay, everyone will gladly turn to them, but until such time bioassay must lead the way.

The word fungicide will be used here in the largest sense, meaning either to kill or inhibit a fungus, or to prevent its effects.

Although laboratory bioassay was used as early as 1807 by Prévost (87) in his classical researches on fungicidal action of copper, the technique has languished unduly. Happily this situation is changing with the realization that rapid further progress in the art must await consolidation of the science.

OBJECTIVES IN THE BIOASSAY OF FUNGICIDES

The techniques of assay, of course, must depend largely upon the objectives that are in mind. Some are interested in discovering a new fungicide. They would probably prefer, first, a screening technique that would throw out unlikely materials.. In our laboratory we have put some 6,000 compounds through such a set of screens of ever growing fineness. The screenings will, of course, be governed by the screen used. One can never be sure that what he throws away as chaff may not contain some grain. One company in screening a series of materials as fungicides discarded tetrachloroquinone (Spergon), but a different screen sorted it out. Such instances have probably occurred before and will occur again. Nevertheless, a screen must be used because most of the material thrown out will be chaff if the screen is reasonably well designed.

Secondly, those interested in developing new materials desire speed. They cannot test several thousand compounds a year in the field, but they can in the laboratory.

Thirdly, they need a method of quality control after they succeed in isolating a new compound because large scale production may have difficulties not encountered in the laboratory where production was in beakers.

One of the limiting factors in screen tests is the number of

organisms that can be used as guinea pigs. Therefore, those interested in fungicides to control specific organisms or mixtures of organisms must test with these. Sometimes different tests must be devised to fit the idiosyncrasies of the organisms.

Moreover, specific uses necessitate other specific tests. Does the material dust well or suspend well in a spray tank? Does it react with other ingredients in the tank? Will it penetrate wood or fabric? Is it volatile at high temperature? Is it toxic to animals?

A few people are interested in the nature of fungicidal action. How do fungicides produce their effect on fungi? Specific techniques may be needed here.

Finally, the farmer, the army or the railroad may like to know how well a material will perform in preventing a plant disease, or in protecting a tent or a railroad tie from decomposing. This last, of course, is a field test and off the reservation for us. We shall attempt to cover the major techniques that apply to the other objectives.

RÔLE OF FUNGICIDES IN CONTROL OF FUNGI

If the derivations of assay techniques are to be grasped, then the uses for fungicides must be reviewed briefly. The time has passed when one can expect a simple overall test such as spore germination to cover the practical uses for fungicides. Chemical fungicides may perform two basic functions in the art of fungus control, prophylaxis and chemotherapy. The purpose of prophylaxis is to protect an object such as a leaf, the human skin or a tent against attack. The object of therapy is to cure an active infection after it has been established. When this is done with chemicals, it is chemotherapy. Obviously, basically different assays will be needed for each. Most assays so far developed apply in the field of prophylaxis or protection. Since many aspects of chemotherapy are new, few techniques are available in that field.

PROPHYLAXIS

Perhaps 99% of current tonnage of fungicides is used for protecting something against fungi. The major portion of this tonnage is applied directly to the object to be protected. Wood is "doped" with creosote to protect it against decay; fabrics are

dunked in copper solutions to make them "mildew proof"; and untold acres of crops are sprayed or dusted until the cuticle is worn thin to protect them against scab, rust, blights and rots of all description. Fungi in their life cycles find themselves open to poisoning at one or both of two points—before inoculation can occur or after inoculation but before penetration.

Preventing inoculation. Despite possible noises from kibitzers, the word "inoculation" will be used here to cover fence posts as well as wheat plants. No other word seems to cover the necessary concepts involved. If chemicals are to prevent inoculation they must be applied to the source of inoculum, whether it be somewhere in the environment of or on the previous host. They may act by killing or inhibiting the fungus or by preventing its sporulation.

Preventing infection after inoculation. Fungicides have played a much wider rôle in preventing infection after inoculation than they have in preventing inoculation.

The majority of protective chemicals are applied to an infection court before inoculation, even though, obviously, they can not act until inoculation has occurred. Wood is creosoted and an apple tree is sprayed before inoculation. In some cases, however, treatment is not applied until after inoculation. Outstanding examples in this field are treatment of wheat seed contaminated with bunt and treatment of contaminated peach buds to control peach leaf curl. In both cases the plant is protected from *infection* even though *inoculation* may already have occurred.

A very special type of prophylaxis merits description. Protectants are now being placed inside a plant rather than outside. Perhaps this should be labelled as "artificial immunization," but, taking the plant's-eye view, it is prophylaxis. Zentmyer (125) has shown quite recently that a small percentage of seedling elms may be thus protected against *Ceratostomella ulmi,* and Stoddard (102) has just shown that peach trees can be protected against the X-disease virus by injecting chemicals into the plants. Clayton *et al.* (13) have suggested that certain glyceride oils appear to immunize tobacco against the downy mildew fungus, but that they do not prevent spore germination. Hence a spore inhibition technique would not have screened out this useful fungicide for that disease.

CHEMOTHERAPY

Although chemical prophylaxis has dominated the field in late years, chemotherapy without doubt was the earliest type of control tried. This was due, of course, to the desire to "treat" a sick plant. One did not worry about plants that were well.

External therapy of powdery mildews with sulfur is probably the oldest practice in plant pathology. It is equally old in human pathology. The use of juglone (5-hydroxy-1-4 naphthoquinone) in walnut husks for fungus ring worm on the skin goes back at least to colonial times.

Internal use of fungicides in plants to alleviate infections is new. Its modern revival as a bona-fide procedure and not a mumbling of quacks probably dates from the work of Howard (47). The Germans have talked much of the "chemotherapeutical index" in their work on cereal seed treatments. Riehm (92) castigates them for the term by pointing out that they were dealing with prophylaxis and not with therapy.

MECHANISMS OF FUNGICIDAL ACTION

Development of assay techniques must be based on the mode of fungicidal action as well as on the uses to which the fungicide is to be put. Four mechanisms of fungicidal action have been uncovered so far. A fungicide may prevent sporulation. Churchman (12) has defined this as "genestatic" because it keeps genesis static. The genestatic properties, of course, can be studied best in culture. Fungicides may inactivate mycotoxins and thus reduce the pathogenic action of the fungus, as Howard has shown (47). This power can be measured in the laboratory on extracts from the fungus, as has been shown (125) for toxins from the Dutch elm disease fungus.

The second two phases of fungicidal action, true killing and inhibition of the fungus, are exceedingly difficult to separate in practice. Usually the two concepts are distinguished by saying that the former is fungicidal action in the sense of the strictest definition and that the latter is fungistatic action. Of course, to the practitioner of the art, it is immaterial whether the fungus is killed or not, but it is of considerable theoretical significance and it often has practical value.

To distinguish between fungicidal and fungistatic, the treated

fungus is placed in a poison-free environment. If it grows again, the action can be assumed to be fungistatic. Time, of course, is a factor (58) in fungistatic value. The longer the fungus is allowed to remain in contact with the fungicide, the less its likelihood, in general, of recovering when placed in a poison-free environment.

Protectants, by definition, must be applied to and remain in the infection courts. They must, therefore, be insoluble in water and have other qualities of resistance to weathering if they are not to be removed before thay can fulfill their function. Horsfall, Marsh, and Martin (46) have divided protective action into the dose factors which determine quantitatively the amount and distribution of the chemical, and the factors for fungicidal value which determine qualitatively the ability of the chemical to inhibit the fungus.

Dose factors can be studied in the laboratory as exercises in physics. They are concerned with deposition, retention, adherence and tenacity, all problems in logistics, to use a current military idiom. They involve getting the materials to the proper place at the proper time.

The factors in fungicidal value can be studied in the laboratory as exercises in chemistry. They are concerned with availability, *i.e.*, the speed with which killing concentrations can be extracted from the insoluble residue. They are also concerned with imherent toxicity, *i.e.*, the ability of the solubilized toxicant to kill or inhibit the fungus.

ASSAYING THE PHYSICAL FACTORS IN FUNGICIDAL ACTION

Clearly a fungicide must be offered to a fungus if it is to kill it. This is not as easy as it seems. Many failures of fungicides result from failure to reach a vital part of the fungus with a disabling dose. As the old proverb goes, "There's many a slip 'twixt the cup and the lip".

In dealing with the physical factors one must consider treatment of a surface as of a leaf or a porous solid such as soil or a piece of wood. As Martin (69, 70) has suggested, many dose factors can be determined by physico-chemical methods, but they are also amenable to bio-assay.

Treatment of Surfaces

Farmers generally treat surfaces. They spray apple foliage and dust wheat seeds. Practical men have a habit of saying that apple scab, for example, is controlled by spraying with sulfur at the rate of 5 pounds per 100 gallons. Apple scab is not controlled by the mixture in the spray tank. They forget the "slips 'twixt cup and lip". They forget the masking effects of foliage, the run-off or the drain-off. Apple scab is controlled by the sulfur that lies on the few square microns of leaf where each spore falls. The problems of placing sulfur on that area and keeping it there are many and varied, but they must be reckoned with in an assay technique.

The three physical factors that govern the effective dosage on a surface at any time are deposition, coverage and tenacity. Deposition is the process of applying the fungicide. Coverage is the uniformity and completeness of distribution of the fungicide over all the areas to be treated. Coverage is often loosely used in the sense of deposition, but it should specifically refer to uniformity and completeness of distribution. Tenacity is the factor that is of peculiar importance to protection. Tenacity is the property of a fungicide to resist removal by weathering (2). Weathering is considered to be any climatological factor operating to reduce a deposit. If the fungicide does not resist strongly the subtractive effects of weathering on the dose, it does not succeed as a protectant.

Choosing the "standard" surface. As yet no one has been able to mimic precisely a leaf surface in the laboratory. Neither has such a necessity been demonstrated. A reproducible surface is more necessary than a so-called natural surface. Some (67) have used leaves as surfaces for germinating spores, but this technique is dreadfully laborious. Others (64, 120) seem to prefer plain glass. The glass surface, however, is not very reproducible. Apparently its surface tension varies widely. Evans and Martin (18) have suggested cellulose nitrate on glass because it is reproducible. This surface has been widely used.

Deposition. Four procedures suggest themselves for applying toxicants to surfaces—pipetting, dipping or spraying the liquids and dusting the powders. If the toxicant is water-soluble, it may be pipetted directly to a surface. Inasmuch as different materials

may affect differently the interfacial tension between water and the surface, they will cover different areas and thus introduce an error. Montgomery and Moore (72) etched circles on glass to prevent spread of the drops, but that does not work too well. To prevent unequal spread of drops, Peterson (83) cemented microscope cover slips to glass and applied his materials to these. Cavity slides are useful for the purpose, but if the material is allowed to dry, it may be concentrated at the edge of the cavity by the meniscus effect. If a spore suspension is used, it may be added directly to the fungicidal liquid in the cavity slide in such volume that it just fills the cavity. This will obviate the optical difficulties of the usual drop of spore suspension and it will often eliminate the running of spores to the center of the drop. A micro-pipette or a graduated hypodermic syringe makes a good applicator.

Dipping has not proved suitable as a means of deposition primarily because of the effects of surface tension. Some materials cling better than others to the dipped surface and hence they will show more potency than the fungicidal properties warrant.

Spraying has been almost universally used as a means of depositing liquids on surfaces. Two basic types of sprayers have been used—horizontal sprayers aimed directly at the target and settling towers. In general, air-operated atomizers, sometimes euphemistically called "air brushes", are used to deposit the materials, although occasionally hydraulic sprayers similar to field models are used. Frear (26) has compared the two as horizontal sprayers and found that the atomizer gives more reproducible data than the other. He offered no explanation for this curious phenomenon.

The target in horizontal sprayers has been variously arranged in order to produce different levels of deposition. Frear (26) put the target on a large revolving wheel which passed it in front of his atomizer. He varied deposit by varying the number of revolutions. Hockenyos and Erwin (40) placed a pendulum shutter in front of a stationary target so that they could vary the deposition by varying the number of "exposures". Evans and Martin (18) used a similar apparatus. Horsfall, Marsh and Martin (46) used a guillotine type of shutter in front of the nozzle. McCallan and Wilcoxon (64) used a shut-off in the air line to regulate spray time.

Since the atomizer depends on a column of moving air to carry the spray droplets, the stream is soft. It wavers in the laboratory breezes and tends to be turbulent. Moreover, the spray fluid tends to be evaporated in transit, not only by contact with dry laboratory air at the edges of the column but also by the dry air used to drive the column. Part of these drawbacks have been avoided by placing the whole affair in a humidified hood (41). Recently a modification has been added in that the compressed air is bubbled through a washing tower. The spray stream is preferably enclosed in a tube to prevent wavering by outside breezes. Probably, however, the tube increases turbulence (86). If so, additional attention should be given to this factor.

It has been demonstrated (112) that spray droplets accumulate an electric charge as they are formed at the nozzle. Moreover, they charge the tube that they are sprayed through, and this produces variation in deposit. If, however, the tube is made of metal and grounded, the charge is drained away and the reproducibility of deposits is improved (86).

The settling tower is a standard tool in some laboratories (64). It was probably first used for insecticide work (104), and has been claimed more precise than a horizontal sprayer (64). It takes much more time, however, because each of the multiple doses must be weighed, and sprayed separately rather than varied through spray time.

Horizontal laboratory dusters have never been designed to equal the precision of horizontal sprayers, but the settling tower type (32, 38) has given good results. The size of the deposit is governed by the size of the load placed in a specially designed narrowly funneled cartridge. The charge is blown upward into the tower by means of compressed air. The settling rate of the dust follows the logarithm of time.

Coverage. Coverage, as already defined, is the uniformity and completeness of deposit, not magnitude of deposit. A deposit can be large but need not necessarily be well distributed. Presumably the best coverage obtainable of any deposit is random distribution of the particles thereof. Dusts distribute themselves over a surface at random according to the Poisson distribution (118). Sprays, however, do not give random coverage. They give blotchy coverage because the particles of toxicant travel, not in-

dependently, but in droplets. As yet no one has developed a chemical assay for coverage. One has had to be content with visual observation or photographs of the uniformity of distribution of deposits. Recently a bioassay of coverage (45) has been developed.

It appears that application of experimental deposits by spraying must be used with discretion. A given mean deposit per unit area will show more potency if applied in weak concentration for a long time than if applied in strong concentration for a short time (45). This fact has shown up only recently as technique has improved.

Surface tension of the spray fluid and of the surface affect coverage. Normally capillary-active materials are added to spray fluids to reduce surface tension and thus to improve coverage. Few realize that opposing factors operate in using such materials. It is true that any volume of liquid containing a surface-active ingredient will spread over more surface than one without. It is equally true, however, that the same ingredient will reduce the size of drops formed by and emitted from the sprayer. In fact, one of the common measures of the effect of a soapy substance on surface tension of a liquid is to count the number of drops formed as one milliliter of liquid issues from a pipette.

The small spray droplets of the liquid with a soapy ingredient will travel more slowly to a target than the larger drops of untreated liquid, and hence the deposition per unit of time will be reduced.

It follows, therefore, that the final coverage obtained on a sprayed surface will be the resultant of three forces which will vary with the surface-active ingredient used—the spreading action of the liquid over the surface, the size of the droplet that arrives on the surface and the number of droplets that arrive per unit of spraying time. Obviously, the interaction of these three factors may bias the result from two materials which differ in the amount or quality of surface-active ingredient.

If the surface tension of two spray fluids being compared differs, the results may be biased in another fashion. The drops of spore suspension applied to the sprayed surface will spread to different sizes and expose different amounts of toxicant to the spores, thus altering the results.

Adherence and retention. The magnitude of the initial deposit depends upon the property of the fungicide to adhere to any surface and upon the property of the surface to retain any material.

This matter is seriously confused in the literature for three reasons: (*a*) because initial adherence is not clearly separated from resistance to weathering, (*b*) because the property of the fungicide to cling has not always been distinguished from the property of the surface to hold, and (*c*) because our language seems to have too few available verbs. Latin provides us with two verbs, "adhaereo," to cling, and "reteneo," to hold back. From the first verb we get "adherence" and "adhesiveness." From the second we get "retention" and "tenacity." These four nouns have been used almost synonymously, or at least each has been applied to all concepts (2, 18, 21, 26, 46). Despite an earlier paper (66), somewhat at variance, it is now proposed that "adherence" (from "adhaereo") apply to the property of the fungicide to cling to the surface, and "retention" (from "reteneo") apply to the property of the surface to hold the material.

These two factors are not to be confused with resistance to weathering which is defined as tenacity (which also comes from "teneo" but which has acquired a connotation of resistance to removal rather than simple ability to cling). Fajans and Martin (21) have used "retention" to apply to the sum of adherence and retention. Frear (26) used "retention" to cover resistance to weathering or tenacity.

Since the concepts have not been too clear, little has been done in the way of devising techniques to separate them. Theoretically a surface is required to measure adherence and a fungicide is required to measure retention. Actually both are probably required for each. Usually the ultimate deposit has been measured. The difference between fungicides can be appraised by using a constant surface, and the difference between surfaces can be appraised by holding the fungicide constant.

Hilgendorff (39) analyzed wheat grain for toxicants present after treatment and showed that wrinkled seeds retain less dust than smooth seeds and that small particles of copper carbonate adhere better than large particles. Fitzgibbon (25) treated seeds with a known weight of material, dropped them through a long

glass tube onto a sieve and weighed them again to determine how much was retained.

The adherence of dust to surfaces seems to be related to electrostatic charges which Bobkov (9) has attempted to measure.

Tenacity. The factors that cause diminution in size of protective deposits are growth of the surface, scrubbing and leaching action of rain, base exchange between dust or dirt and the deposit, and sun action. Dusts may be jarred off or blown off by wind. Exceedingly little has been done on any weather factor except water. Probably it is the most important.

Tenacity, being a quantitative dosage factor, can be assayed by chemical means, provided a reagent is known for the fungicide used and provided interference with other ingredients is not serious. In such cases bioassay will be necessary. Martin (70) has said that no one has shown that bioassay of tenacity of protectants produces any more useful results than chemical assay. This is doubtless true for different samples of the same material, but Miller (71) has shown that comparisons of the tenacity of "fixed copper" materials are made more accurately through bioassay than through chemical assay.

A few chemical determinations have been made of residues on growing apple fruits. The data are inconclusive. It would seem interesting to treat a flexible surface like thin rubber and then stretch it to see what changes in fungicidal action would be induced. This would then probably turn out to be a study in coverage.

Although few studies on base exchange have been made, it has been shown here that some soils do affect fungicidal deposits, especially deposits of the relatively unstable organic fungicides such as disodium ethylene bisdithiocarbamate. It may be that low-growing plants accumulate enough dirt on their leaves to affect seriously the performance of fungicides. Tetrachloroquinone on seeds tends to be inactivated in alkaline soils. Cuprous oxide on seeds, on the contrary, tends to be solubilized in acid soils so that the injury factor is increased.

Kraus (51, 52) approximated a measure of the effect of soil on the action of protectants by germinating spores of *Tilletia tritici* on slate dust saturated with lime water. Tornow (107) claimed that the refractory germ tubes of the fungus show up

clearly against the dark soil, especially if talc is mixed with the soil.

Sunlight produces photochemical effects on many chemicals. As far as known now, it has little effect on deposits of Bordeaux mixture, but it will no doubt prove increasingly important as more organics are introduced into practice. Tetrachloroquinone already has failed as a foliage fungicide on account of photochemical effects, even though it has succeeded very well as a seed protectant when used in the dark below ground.

Some fungicides do volatilize slowly and hence lose potency slowly. Sulfur sublimes on the foliage, and the chlorinated phenols volatilize from wood and cloth. Thorburn and Vincent (106) suggested that treated fabrics be placed in front of a fan for seven days and then assayed with the test fungus. As far as known, no one has ever made similar tests on sulfur, but it needs doing.

The effects of rain on fungicidal deposits have been studied extensively since the days of Girard (30) 50 years ago. He used artificial rain on sprayed foliage. Some tests are based on simple soaking of the deposits in water. The Heuberger (37, 121) technique is more. It involves passing the treated surfaces rapidly through water to simulate the thrashing and soaking action of rains. Study of some of his data has shown, as expected, that the deposits come off in accordance with the logarithm of the number of times they are washed. From that the slope of the wash-off curve will serve as a criterion of resistance to rainfall. It serves much better for precision work than the proportion of deposit removed by a constant amount of washing, which is the usual standard. Mechanical washers (72) have been devised, and they are probably to be preferred to Heuberger's hand test. Sometimes (118) a rain test using water from a suspended nozzle has been used, but it probably is not sufficiently different in action and is very wasteful of distilled water.

Nikitin (77) felt that he could measure in the laboratory the resistance to weathering of copper protectants by measuring their adherence to the diaphragm in an electrodialysis cell.

Eidman and Berwig (17) showed that the loss of dusts by wind action seemed to follow a different course from loss by jarring and that resistance to rain seemed to follow still a different course. This suggests that the factors that govern each

differ. Doubtless, electrostatic charges are involved in prevention of loss by jarring, whereas they would play little part in loss by rain action.

Impregnation of Solids

Solids often must be impregnated with fungicides, and methods for accomplishing this are available. The solids usually concerned are woods, fabrics and soil, and the general techniques are dipping or soaking and vacuum and/or pressure.

Dipping or soaking. Perhaps the best example of soaking is the bath treatment of fabrics, the laboratory examination of which has been discussed recently (28). In another type of test the fabric is sprayed with the chemical to be tested (106).

Vacuum and/or pressure. Waterman et al. (113) have described a laboratory process for impregnating experimental wood blocks. They are placed in a container under a bell jar which is then exhausted to 2 cm. of mercury. Enough liquid is next introduced into the container so that the blocks are submerged when air is admitted. In commercial practice the vacuum treatment may be followed by or substituted for pressure.

Fumigation

Fumigation is sometimes used to introduce gases into solids such as the interior of a plant or soil. Techniques for evaluating fumigants have been described (20, 84, 85, 111). Entomologists also have worked extensively to develop procedures for measuring toxicity of fumigants.

ASSAYING THE CHEMICAL FACTORS IN FUNGICIDAL ACTION

Having examined the methods for applying fungicides, it is time to examine the methods for measuring the potency of the chemicals applied. Such methods range all the way from big field tests with large commercial equipment down to micropipettes and cavity slides for a microscope.

Some five laboratory or greenhouse tests have been described that give more or less information about fungicidal potency. Those adapted to the laboratory are spore germination, electrical resistance, and growth on agar, wood blocks or fabrics. Those adapted to the greenhouse are soil burial tests and infection of host plants.

Spore-germination Test

The spore-germination test may be conducted in two ways—on natural surfaces or on artificial surfaces. Tests on natural surfaces have been developed only lately and are not very useful because of the difficulty of seeing the spores. Marsh (67) germinated spores on treated apple leaves and then cleared the color from the leaves for microscopic examination. Zade (124) inoculated oat seeds with *Ustilago avenae*, treated the seeds with chemicals, removed the glumes and examined the adhering spores for germination.

The spore-germination test on artificial surfaces has been heavily investigated. Prévost (37), the pioneer laboratory researcher, used it. Carleton (11), 50 years ago, used the spore-germination test as a screen for a large number of possible toxicants for cereal rust, and Swingle (103) used it in one of the earliest studies ever made on the mechanism of action of bordeaux mixture. Although many laboratories have dabbled with it, the laboratory of plant pathology at Cornell has kept everlastingly at it under the direction of the late Professor H. H. Whetzel (116). Reddick and Wallace (89) started the work in 1910. Later workers, especially McCallan (56), carried the ball during years when laboratory assay of fungicides was in low repute. In recent years work in England (46, 67, 72) has contributed to the perfection of the technique of assay by spore germination. German workers do not seem to have paid much attention to Schmidt (95-98) who 20 years ago published rather complete techniques for laboratory assay of fungicides by the spore-germination technique.

The Committee of the American Phytopathological Society on the Standardization of Fungicidal Tests has studied the spore-germination technique and has published instructions on its use (2). Rangel (88) examined the techniques of various phytopathologists and tentatively adopted the spore-germination technique as the most suitable.

The spore-germination technique is rapid. An experiment can be set up one day and data taken the next. Aseptic precautions are required only to produce the spores. The tests can be conducted without the complications due to foreign matter inherent in agar and broth, but if foreign matter is significant, it can be

included at the behest of the experimenter, and, therefore, its effect can be measured.

The spore-germination technique is currently enjoying a wide vogue in laboratories, both State and commercial, where new fungicides are being developed. It can screen enormous numbers of new materials and it then serves admirably for quality control on the manufacture of a new material before chemical methods are devised. Oftentimes the spores can tell things about the new chemical that no other test can reveal.

The spore-germination technique is especially well adapted for work on prophylactic chemicals, but it is useful in the early stages also for therapeutic chemicals that are effective because they kill or inhibit fungi. The test is worthless, of course, where toxin inactivation is important or where prevention of sporulation is important. The technique can be used to measure both fungistatic and fungicidal power, using these words in the sense of inhibiting or of killing the spores, and as generally used, it measures fungistatic power because usually the spores are left in contact with the toxicant. This is of no serious import to strictly protective chemicals because by definition they must remain in the infection court.

Measurement of fungicidal power can be accomplished simply by removing spores after a given time from the toxicant and washing them as free of toxicant as possible. This is the standard procedure for bactericides. The bacteria in standardized numbers per volume of liquid are pipetted into tubes containing the toxicant. An aliquot is removed after suitable time intervals and plated to determine the percentage killed. McCallan and Wellman (58) have treated spores similarly, except that they centrifuged the spores out, washed them, and counted them after they germinated in distilled water.

The fungus. Fungicide researchers would laugh and sing if it were always feasible to use the organism they were interested in as a test fungus. Use of another organism causes them to worry about the possibility of differences in susceptibility (50, 61). Unfortunately it is seldom possible to use one's private fungus in the spore-germination test. *Venturia inaequalis* has received much attention in the field. It practically refuses to sporulate in the laboratory despite numerous dodges that are

said to be successful (72, 79). Sporangia of *Phytophthora in-festans* are difficult to handle. Rusts refuse to grow in culture, and smuts refuse to produce chlamydospores. *Penicillium* and *Asper-gillus* spores are too small to be seen readily. For these reasons two organisms at present are carrying the major load—*Sclero-tinia fructicola* and *Macrosporium sarcinaeforme*. The former produces a much larger volume of spores in a shorter time than the latter, but it requires a "shot-in-the-arm" to make the spores germinate properly. Orange juice (119) is most commonly used. The latter fungus has big black spores with hyaline germ tubes, easy on the eyes to read. This becomes important to the day-by-day operator. Sometimes *Alternaria solani*, *Glomerella cin-gulata* and *Rhizopus nigricans* are used, but seldom. Only *S. fruticola* is sensitive to elemental sulfur, although the others are sensitive to organic sulfur fungicides.

Obtaining spores. Potato agar slants are suitable for produc-ing spores of most species, although *M. sarcinaeforme* sporulates better on oat agar. Usually the spores are harvested for use as soon as the slant produces enough. About seven days are re-quired for *Sclerotinia* and 14 days for *Macrosporium*. The spores are washed from the slants in double distilled water which obviates possible toxic contamination in the water. Some operators (63) prefer to centrifuge and wash once to get rid of nutrient in the spore suspension. That probably is necessary for the highest precision, but it is not followed in our laboratory.

Hamilton and Weaver (36) resurrected an old bacteriological dodge (75) by freezing spores in summer when they can be ob-tained in the field and thus preserving them for later use. This technique has merit for such desirable species as *Venturia in-aequalis*.

Resistance of spores to poisoning decreases with age (10, 14); hence age should be standardized. It is interesting that house flies also become more susceptible to pyrethrum as they grow old (100).

It has been shown that the percentage of spores inhibited fol-lows the logarithm of the dose of toxicant per spore (44, 61). Hence it is necessary to control the density of spores in the suspension. The Fuchs-Rosenthal haemacytometer (62, 120) has been recommended, but in our laboratory we find that it has

big errors because large spores, especially those of *M. sarcinae-forme,* do not act like bacteria; they are too sluggish in traveling into the counting cell, and hence the density is underestimated. It seems preferable to return to the earlier method of regulating the density by the number of spores per low power field of the microscope (10 $\times$ ocular, 16 mm. objective). Forty spores per field is a convenient number. Of course, standard procedure should be adopted in making the drop. The surface should be standard. The pipette should be standard. Uniform numbers in all drops can be obtained by bubbling air through the pipette used (80).

Spores of several species of fungi will not germinate when freed of nutrient.

It is imperative that spores germinate well. It would be eminently desirable not to have a single spore refuse to germinate because it is impossible to separate spores that are killed by the fungicide from those that are so ornery as not to germinate. Statisticians have almost tied themselves in knots trying to calculate a correction for "natural mortality", but as yet no one seems to have derived a wholly satisfactory answer (1, 24). Perhaps the greatest advantage in using *M. sarcinaeforme* is that it usually germinates almost 100%.

For those spores that do not germinate well, something must be added. McCallan and Wilcoxon (62, 63) have found that ultra-filtered orange juice from a frozen stock is suitable. Miller (71*a*) has just shown that citrates perform the same function. Sometimes biotin or coenzyme R (31) has been used. Lin (55) contended that the necessity for an added material is primarily one of energy requirement, but the work with biotin suggests that it is a vitamin shortage.

Applying spores. Drops of spore suspension are applied in duplicate to the sprayed surfaces. Of course, care should be taken to apply uniform drops, or else the dose relations will be disturbed. Drops of 0.05 ml. in volume have been standard (2). As already discussed, the drops may spread to different areas on deposits that contain wetting agents. Therefore, it may be necessary to restrict their spread with an etched ring, by using a cavity slide, or by other dodge.

The sprayed surfaces with their drops of spores are placed on

racks in large moist chambers (250 mm. D.) or so-called culture dishes. The racks may be made of glass tubing or, better still, of some metal like sheet aluminum. The chambers are inverted for use so that they can be sealed with a water seal. Since some fungicides may be slightly volatile, it is wise to have a check slide in each chamber. If volatile materials are being tried, it may be necessary to put each slide in a separate Petri dish moist chamber.

The spores are incubated usually overnight at the optimum temperature for the fungus, since the fungus is most difficult to kill at its optimum temperature (87). When germination is complete, the slides are removed and counted under low power magnification of 100 or 150X. A cover slip is not required.

The time for counting germination is more important than is usually admitted. Martin (68) pointed out that to make counts when the untreated spores are all germinated might be misleading. Later Dimond et al. (16) showed experimentally an influence of time, and this was confirmed by Wellman and McCallan (114). It is clear that spore germination in all treatments must be allowed to proceed to completion.

The novice usually counts more spores than necessary. Normally it is sufficient to count 50 spores in each of two drops. Since the results are influenced by natural mortality, Wellman and McCallan (114) have suggested a simple correction for spores of high viability. They suggested that as many extra spores be counted as the check is short of 100. For example, if the check gives 98% germination, then 51 spores should be counted in each drop to make a total of 102. The two are then subtracted from the ungerminated results.

The question always arises in using the spore-germination technique, whether the length of the germ tube should be determined in addition to the percentage of germination. Of course, if the length of germ tube is recorded, special statistics would be needed for evaluating the data. Hamilton et al. (34), without giving data, say that "the relative length of germ tubes is a better criterion of the inhibitory action of sulphur than the percentage of spores germinated". Anderson (3) made an extensive study of the two methods for measuring the inhibitory effect of wheat extracts on germination of rust spores. Apparently the two do

not bear linear relations to each other, but rather some sort of hyperbolic relation. There was no evidence that one was qualitatively different from the other. Therefore we must deduce that either is suitable but that measuring germ tubes will probably not give results qualitatively different from determining the percentage of germination. It was the opinion of the members of the Committee on Standardization of Fungicide Tests (2) that germ tube length is not of enough importance to warrant the extra work.

The Electrical Resistance Test

For many years Osterhout (78) has pursued the theory that the effect of many kinds of substances on cells can be measured by electrical resistance of the treated plants. He has confined his attentions largely to lower green plants, but one of his students (65) took a flier into fungi and fungicides. Her technique has never received much attention among the clan of fungicide researchers, perhaps because the technique seems remote from everyday experience, perhaps because she rated mercuric chloride less efficient than copper sulfate, an idea contrary to most opinion.

Growth on Agar

One of the most extensively used tests is that in which the toxicant is mixed in agar and the fungus is planted and allowed to struggle along on such poisoned food. The technique has been used mostly by those interested in wood preservatives. An extensive literature on fungicidal action has accumulated through its use.

Historically it seems significant that the spore-germination test should have been developed primarily by plant pathologists interested in protecting foliage where spores constitute the inoculum, whereas the agar test, using mycelium, has been developed by those interested in wood preservation where mycelium constitutes the inoculum.

When the two are considered side by side, it is clear, however, that the spore-germination test has great advantages in speed, accuracy and freedom from contamination by adsorptive colloidal material. It will probably therefore gradually replace the agar test where the objective is a study of fungicidal action. The agar

test will probably persist only for him who is concerned primarily about specificity of organisms and who cannot use spores of the organism concerned. He will probably reason that he would rather use his own rather than another organism, despite the disadvantages of the agar technique.

The agar test probably runs back historically about as far as solid media. It became quantitative when Falck (22) showed that the radial growth of a fungus in Petri dishes is linear with time and that the presence of a toxicant in the agar does not alter the fact. It then became possible to measure the toxicity of a compound by the number of days required to reach a particular diameter of thallus, or by the diameter of a thallus reached in a specified number of days.

The technique has reached its best performance at the Forest Products Laboratory in Madison, Wisconsin (4, 99). In brief, it involves mixing the toxicant with agar, usually malt agar, just before the agar hardens. A uniform quantity of treated media is poured into a standard Erlenmeyer flask. Upon inoculation the flask is stoppered to prevent loss of material and water by possible volatilization and to avoid cross transfer between flasks of volatile materials. The flasks are incubated at a standard temperature. Several concentrations of each material are employed. It is customary to use a standard fungus in the wood preservation studies, the commonest being an unidentified strain of *Fomes* which has a potent ability to rot wood. The new method of growing the fungus in a horizontal glass tube half filled with agar would probably be preferable to the flask method because the hyphae may be permitted to grow to greater length (93).

The agar test can be used for measuring genestatic power (12) of fungicides. If certain chemicals such as anthracene derivatives (101) permit growth but prevent sporulation, such chemicals can be listed as genestatic.

Growth on Wood Blocks or Fabrics

British and German workers on wood preservatives have bitterly opposed (23, 54) the agar technique of their American colleagues. The British have contended for the wood block method which they feel is more realistic. They inoculate a block of treated wood and incubate it several months in a Kollé flask. If the

agar technique is slow, the wood block test creeps. The wood block test is perhaps characteristic of foresters and of those concerned with wood problems because they think in terms of years whereas others think in terms of hours or days. Perhaps a wood block test is worth using as a "pilot plant" test, but for study of fungicidal action or for producing new fungicides, it is too slow for real consideration. Not only is it slow, it is not precise. Measurement of fungicidal or fungistatic action in it is not easy, and strength tests of treated blocks or gravimetric measurements of weight loss may be very difficult to evaluate. The wood block test will be discussed again under burial tests.

Waterman *et al.* (113) have defended the wood block method against the agar method because of the artificial character of the dispersion of the toxicant in agar. They have criticized the Kollé flask method, so commonly used in Europe, because the wood block becomes too saturated with water. Their technique is essentially to place the wood block in a moist chamber and to feed water to it by means of a wooden wick. Data are taken by estimating growth on the treated block, by losses in weight and by breaking tests. A similar wood block test in Petri dish moist chambers has been developed for painted surfaces by reading the growth of the *Aspergillus* indicator by a grading system (82).

The growth of organisms on treated fabrics has been used extensively in research on the use of fungicides to protect fabrics. For many years *Chaetomium globosum* was the test fungus (105). Recently species of *Aspergillus* and other genera have been used. Test strips of the treated fabric are placed in Petri dishes on a carbon-free agar base and inoculated with a spore suspension. After incubation for a fortnight, the tensile strength of the treated strips is determined. In other cases (106) no agar is used. Amount of mildew is judged visually.

Soil Burial Tests

The burial test is rapidly forging ahead as a test of protective action. It was probably first used by those interested in wood preservation, but it is currently receiving much attention by those interested in fabric preservation (5). Assay of seed protectants is a species of burial test because seeds are buried when they are planted.

The burial test is a field test in effect, and field tests are out of bounds for this paper. Nevertheless it will be discussed because at present it has advantages not now available in other tests. When treated solids or surfaces are buried underground, they come very close to an environment saturated with inoculum. Results of some sort, therefore, are assured. Whether they can be measured is another matter. If the soil is kept damp, leaching will proceed at a continuous pace, not limited to rainy or foggy periods. Organic matter is present to tie up any protein-loving fungicide, and base-exchange can proceed at a rapid pace. Of course, the type of base exchange will vary greatly with soil type and composition, and it may be unpredictable. A burial test involves the screen with perhaps the finest meshes through which a new fungicide must pass in our series of tests with new materials.

In research on wood preservatives it has been common practice to have stake farms in various areas, especially tropical areas, where treated stakes could be driven into the ground and their behavior observed at intervals. Recently the trend has been toward using much smaller blocks of wood (53) completely buried. The theory is that the smaller the block, the greater the surface area exposed to corrosion per unit weight, and hence the more rapid the destruction.

Greenhouse Tests on Foliage and Seeds

Foliage tests in the greenhouse have been used for measuring both protective and therapeutic powers of fungicides.

During the evolution of true laboratory testing of fungicides, many experimenters preferred to operate somewhere between gross field tests and the fumbling laboratory assay of the time. Keitt and his co-workers (33, 49) set up large scale greenhouse equipment complete with incubation chambers to measure protective action. The trees were sprayed with hand equipment and inoculated with pathogenic fungi. As laboratory assay became more precise it became obvious that more precision was needed in the greenhouse trials if they were to continue to compete. Hamilton and Weaver (35) designed but did not describe accurately their very elaborate and refined equipment for greenhouse assay of fungicides employing potted trees. Nielson (76) has described their equipment in somewhat more detail.

Marsh (67) sprayed leaves on twigs and placed them in a micromoist chamber after inoculation. His technique, in effect, was the same as in most laboratory assay, but he used leaves instead of an artificial surface and found that the "living leaf has proved less flattering to the fungicide than has the slide".

It is curious that McCallan, one of the most enthusiastic supporters of laboratory assay of fungicides, has now published on a method of greenhouse assay (57, 59, 60). With his colleague, Wellman, he proposed the tomato as an easily grown plant and *Alternaria solani* as a not quite so easily grown pathogen. The potted plant to be sprayed is mounted on a turntable. The "paint gun" sprayer with stirrer is mounted on the end of an adjustable arm. Plants are inoculated by atomizing them with a spore suspension at constant density. Data are taken as number of lesions per unit leaf area and expressed as percentages of the check. In another paper (115) the authors discussed the use of wheat smut in greenhouse work and also correlation between laboratory and greenhouse assays. In general, there was reasonably good agreement when it is remembered that the greenhouse assay was shown to be less reproducible than the laboratory assay. The performance of nitrogen tautomers in the greenhouse was not well predicted in the laboratory.

Salmon and his co-workers (19) at Wye in England have done some excellent research using therapy of powdery mildew on hop leaves as a "guinea pig". Hop leaves occur opposite to each other on the stem. One leaf is treated, the other is kept as check. The precision of the test is not very high, but it gave those workers some good data on the relative performance of a wide range of materials. Of course, the technique is not capable of differentiating the fungistatic from the fungicidal property of the test material.

The German workers, beginning with Riehm (91) and Gassner (29), have tested fungicides against wheat smut in the greenhouse. A couple of Ulstermen lately (73, 74) have used oat seeds infected with *Helminthosporium* and flax seed infected with one or more fungi to measure fungicidal potency. Taking heavily infected seed, they coat it with the chemical to be tested and then place 100 seeds on wet filter paper in Petri dish moist chambers. The fungus grows out through the layer of chemical over the

seed within four days if the chemical is weak. At that stage the dishes are irradiated with strong white light to induce sporulation of the fungus (*Helminthosporium*) which can be confirmed by the eleventh day. The percentage of seeds showing sporulation is a measure of toxicity of the coated layer.

Tests of fungicides on foliage and seeds will probably pay dividends until such time as the significance of the host in fungicidal action is known. The host conceivably can play a part in both the dosage and fungicidal value sections of protective value. If the surface is wrinkled, veined or hairy, it will expose much more surface for protection than a plane surface and thus influence unfavorably the dose factors, as Marsh (67) has suggested. It may aid in making the toxicant more available to the spores and hence it may activate the material, as Yarwood has suggested (122).

If the first case is true, then a simple correction for dose difference will be enough. If the second is true, then the whole assay may be disturbed in such a fashion that a simple correction will not be sufficient.

THE YARDSTICK OF FUNGICIDAL POTENCY

The potency of a fungicide is an illusive quality. It must be measured if it is to be understood, but its measurement is as shot through with fallacious thinking as any field of science. Fortunately the measurement of fungicidal potency is clearly akin to the measurement of insecticidal value, of drug value and of almost any value in biology. We can, therefore, make use of the developments in those fields. For the same reason, however, it is almost as easy to be misled by the fallacies in these other fields as it is to be helped by the progress that they have made.

The Two Methods of Measurement

Clearly in bioassay the effect of a fungicide must be measured on the fungus. Two methods of measurement suggest themselves (*a*) to measure the inhibition of growth or spore germination by any given amount of fungicide; and (*b*) to determine the amount of fungicide necessary to produce any given level of inhibition (42). Even to the casual thinker it is clear that the former method involves much less manipulation than the latter; naturally

it has been heavily leaned upon. Modern knowledge, however, shows it to be a frail reed instead of a substantial lamp post.

Measurements of response for a single dose can be made precisely enough, but they may not signify the true difference betwen any two fungicides. Moreover, the technique may sometimes rate two compounds in one order, sometimes in the reverse order (15, 109). The reasons for the anomalous situation will be developed below.

The Dosage-response Curve

If one chooses the method of comparison through the dose required for equal response, obviously he must use a series of doses of each material in each experiment and he must determine the inhibition from each dose. Such an experimental design will result in a series of data from which can be plotted a dosage-response curve. Since dose is the independent variable, it should be plotted on the X axis, and inhibition, being the dependent variable, should be plotted on the Y axis. Data so plotted on an arithmetic grid will give a sigmoid curve. The two halves of the S will not be equal, however. The upper half will be much fatter than the lower, and the upper end of the curve will tail off slowly toward the ceiling of response.

Clearly the relation betwen dose and response is flexible, but since no rubber ruler is suitable, it needs a steel center to straighten it out. If the curve were a symmetrical S, probably only one axis would need to be modified in order to straighten it. The fact that it is not symmetrical suggests that both axes need some tinkering to straighten it.

Action of dose. Reference to elementary biology or physics will recall that many processes of nature follow the law of diminishing returns which means that an increase in stimulus will not result in an equivalent increase in response. Stated in terms of the present problem, it means that an increase in response requires a geometric increase in dose. Therefore the fattened upper portion of the curve is due to the law of diminishing returns. It can be reduced to normal shape by expressing the dose in logarithms instead of in arithmetic units. This process seems to bother some assayers. It should not. They deal almost daily with pH units and these are in logarithms. Of course, they sometimes fail to

appreciate that fact because they make simple arithmetic averages of pH values.

Action of percentage response. Having eliminated the bulbous upper portion of the curve, it is apparent that the S shape must be caused by irregularities in the weight of the values on the other axis. It turns out that this is due to the rubber nature of the percentage scale. The percentage scale is least sensitive in the middle range or at 50%, and its sensitivity increases rapidly as it approaches the ceiling and the floor of response. The percentage numbers remain at equal intervals despite the fact that the sensitivity increases. It is necessary to stretch the space between the percentage values if we are to use the response scale as a firm ruler. This has been done graphically on probability paper and statistically by the use of probits which are so arranged that they measure response in equal steps. Bliss (6, 7, 8) devised the probit as a unit of measure for bioassay, but it was based on a series of earlier studies (*e.g.*, 27, 108, 117).

Having hurried thus through a maze of statistical reasoning in a few words, it can be summarized for most persons interested in bioassays by stating that data plotted on logarithmic-probability paper will give a straight line, which means that the data are now measured by a firm ruler. Data so treated provide two or three useful values, LD 50 and LD 90 and slope.

Significance of LD values. The phrase, LD values, sounds like a code message from the hero in melodrama. Actually it is shorthand for lethal dose for any given level of response. Thus LD 50 means the lethal dose for 50% response and LD 90 means lethal dose for 90% response. The derivation of these values stems back to Trevan (108). LD values are mainly a measure of fungicidal potency (16, 69), especially between samples of the material or between materials with the same mode of action. One can say with other words that LD values measure the availability factor in fungicidal value. They are wonderfully useful, for example, in quality control for new material. They tell very accurately what the relative performance of any two batches is. LD values are excellent for studying differences in particle size of any compound (69). LD values are indicative when used for two different materials, but they may be heavily influenced by the slope of the dosage-response curve, as discussed below.

Significance of slope. The angle that the straightened dosage-response curve makes with the horizontal is slope. It can be determined by calculation, but it is most easily determined by a protractor if the line is plotted on a standard logarithmic-probability grid.[1] Slope of the dosage-response curve is becoming more interesting all along as a characteristic of fungicides. It gives a dynamic picture of events. It has been made the subject of two extensive papers (16, 61). Slope can never be obtained, of course, from the static experimental design where single doses are used.

First, slope is a characteristic of the fungicide itself. Different fungicides may have different slopes. In general, slope is considered to be an indication of the mode of toxic action. If the slope differs between two compounds, it is a fair experimental assumption that they differ in mode of action. Slope, therefore, measures inherent toxicity of that portion of the material that is available (16, 69). Parker-Rhodes (81) has greatly extended the theory that slope measures inherent toxicity. He has made much of the fact that some other function of the concentration than the logarithm may be necessary to produce a linear regression line.

If, however, two materials of interest do differ in slope, then it follows that the lines must cross somewhere. If they cross, then it follows that for doses above the point of crossing, one compound will be more potent than the other, but below the point of crossing they will be reversed. Since environment probably changes the point of crossing (109), it is plain that single dose research might well show the compounds to be different in two separate experiments or in two separate areas.

Clearly in this case LD values also may be misleading. The lethal dose would be inverted for the two materials, on both sides of the crossing point. In such cases it is coming to be accepted that comparisons through some high LD value such as LD 90 are to be preferred. Such comparisons will minimize the effects of different slopes.

Recently (45), it has been discovered that slope may be a measure of coverage. The more nearly random the particles of toxicant are when exposed to the spores, the steeper the slopes will be. Therefore, if two materials seem to differ in slope, it

[1] We use grid No. 3128, made by Codex Book Co., Inc., Norwood, Mass.

would be well to ascertain whether they are exposed equally at random to the spores, or if the spores are exposed equally at random to the toxicant.

McCallan and Wellman (58) have reported that the slope of their fungistatic curve was steeper than that for the fungicidal curve for the same water-soluble chemical. This may have been due to a coverage factor in their technique. In the fungicidal technique the spores were soaked in test tubes for predetermined lengths of time in the solution of toxicant, then centrifuged and washed free of it. In the fungistatic test the liquid was placed in drops on glass slides. In both cases the number of spores per cubic centimeter was a constant. Therefore in the slide test with small drops of spore suspension, the spores probably settled onto the slide at random and were exposed almost at random to the poison. In the test tubes for the fungicidal test the spores, even at equal overall concentration per cubic centimeter, probably settled in a mass to the bottom of the tube, where each spore was clearly not exposed to as much toxicant as was each spore on the glass slide of the fungistatic test. Therefore the kill was not so high as expected. Since the position of the spores remained constant irrespective of concentration, it follows that the killing differential probably increased with concentration and the slope was flatter for a test in a tube than for a test on slides.

Slope is related to age of spores. Spores from old cultures produce flatter slopes than spores from young cultures (16). Ipsen (48) shows that the same phenomenon occurs for red cells in blood. Addition of growth promoters like orange juice (16) steepens the slope, possibly because the growth promoter may cause old spores to act more nearly like young ones.

Value of a Standard Fungicide

It is common practice in any procedure to carry a standard treatment as a reference point. In colorimetric chemical analysis, the color of the unknown is compared with that of the standard and thus the value of the unknown is determined. In toxicology also it has been common practice to refer the performance of the test toxicant to the standard.

The famous phenol coefficient so well known in bacteriology is aimed to provide such a reference comparison—phenol, of

course, being the reference. The phenol coefficient was intro-
duced by Rideal and Walker (90). Young and Cooper (123)
proposed a copper sulfate coefficient for fungicides, and later a
Bordeaux coefficient was introduced for comparing protectants
(44, 120).

We now know that such coefficients have been greatly over-
rated. They have been used in cases where they should never
have been used, and they have led inevitably, therefore, to er-
roneous conclusions. The phenol coefficient, for instance, has been
used for rating such divergent substances as nitrated derivatives,
quaternary ammoniums and organic mercuries. Such a procedure
is equivalent to using a copper sulfate standard in a colorimeter
to measure mercury, amino acids and sodium nitrate. It is not
astonishing that phenol coefficients come in for bitter denuncia-
tion at times, especially by the practical user of bactericides.
Likewise, Bordeaux coefficients can be misused.

Bliss and Marks (8) have pointed out that the slopes of the
dosage-response curves must be parallel if the coefficient is to be
sound. Obviously if the slopes are not parallel, the coefficient
will be different for each level of comparison. The explanation,
of course, is still more fundamental. Since slope signifies mode
of toxic action, other things being equal, it means that materials
of different slope are acting differently and hence are not refer-
able to the same standard.

The argument, like many others, breaks down if carried too
far. Clearly the tensile strength of steel differs from that of
lead, and copper oxide is more fungicidal than zinc oxide. These
two statements in effect indicate quantitative differences of great
significance to a bridge builder or a farmer. The differences
can be calculated in terms of ratios or coefficients. In the case
of steel it is the relative breaking strengths. In the case of
fungicides it should be the relative LD 90 values, since LD 90
is high in the scale of potency but not so high as to experience
too much experimental flutter towards the ceiling.

The growth of the concept of a standard can be followed from
the work at Boyce Thompson Institute. At first (120) they fav-
ored the standard and a coefficient as a means of reducing day-to-
day error. Later they reaffirmed their interest in the standard
(63), but by 1941 they receded somewhat from their enthusiastic

position when they realized that slope made a big difference. They agree that "a standard may be employed as a check on the reproducibility of the technique and for the purpose of orientation in preliminary tests of new compounds.   Likewise a standard may be used effectively to adjust the replicate test variation provided the compounds are essentially similar in chemical composition and slope" (61).   In effect they agree that a standard is useful for appraising the impact of differences in resistance level (44) of spore populations from day to day.   Part of their argument against the standard is that slope differences may throw it off if the comparison is at LD 50.   Much of this argument fails if the coefficient is based on LD 90.

Biological Variation

The old bogey of biological variation crops up in fungicidal assays as in all similar assays.   Even if all manipulative errors could be eliminated, experimenters would still be confronted with a residue of error known as the "error of sampling".   This paper is no place in which to jump into the statistical intricacies of that subject, except to touch briefly on how it affects fungicidal assay.

The best technical discussions of the subject as it applies here can be found in papers by Bliss and Cattell (7), Ipsen (48) and Wilcoxon and McCallan (120).   Wilcoxon and McCallan (120) have shown that most usual calculations of variation can be made graphically from the curves as plotted on logarithmic-probability paper, as already discussed.   Not only can the error be determined but also Chi square can be determined from the graph by means of a nomograph which they give.

Parker-Rhodes (81) has developed a new theory of statistics for toxicity assays in which he makes use of an index of variability of spores to a toxicant.   This index he calls "alpha".   Correspondence and conversation with eminent statisticians in the field of toxicology reveals that no one has yet converted the implications of alpha to words of one syllable; hence it needs little more than passing mention in a general discussion.

Recently McCallan and Wellman (57, 59, 60) have made progress in statistics of using dosage-response curves on foliage diseases and spore germination.

The Threshold

One very widespread concept in toxicological research is the threshold dose for toxicity. It is a concept covered with confusion and it has therefore often impeded about as much progress as it may have fostered. Much of the befuddlement arises because the response of an individual to a poison is confused with the response of a population. Bliss and Cattell (7) speak of the threshold dose of an individual. This dose is admittedly difficult to determine experimentally and hence the "threshold" selected is admittedly artificial. Nevertheless a reasonably satisfactory judgment of threshold dose can be derived for the individual.

A spore may produce only gnarled tubes at one dose. It may be able to push out only knobs at the next higher dose and show no tube at the next. If the temperature of testing is changed to a more suitable level, these grades might be pushed up a notch so that one still higher dose is required for each response. Just which dose then is the threshold dose? One has to be chosen arbitrarily.

Still one might not wish to quibble about the threshold dose of an individual, however worthwhile it might be in precision work. For that reason the "killing point" (4) of a wood preservative on a single fungus thallus is probably defensible, although it would probably be easier to determine experimentally the dose for 90% inhibition than for 100% inhibition.

To use threshold dose for populations is a dangerous procedure because it may lead the experimenter into an indefensible position, primarily because of variation among individuals. The dose to kill the most susceptible individual is perhaps the threshold dose, but even the individual may already have been killed by "natural mortality". In practice no dosage curves are known to have been published wherein no individuals died at the weak doses. Wadley and Sullivan (110) contend for a threshold dose and yet their own curves taper off at the bottom. If there were a threshold dose for the population of flies they were using, the curve should have dropped precipitately to zero at that mythical dose. The curve did not; it merely approached zero asymptotically, just as other curves do.

Salvin (94) speaks positively of the threshold dose of an antibiotic, but his curve is smooth, tapering off towards zero with no precipitate drop to zero at the hypothetical threshold.

SOME PRACTICAL PREDICTIONS ALREADY MADE

An accelerated assay of fungicides can hardly be expected to take hold unless it "pays off". Laboratory research is paying off. It has been demonstrated (43) that a knowledge of fungicidal value and tenacity is useful in predicting the field performance of "fixed copper" fungicides. If spore-inhibiting property is equal, materials separate themselves in accordance with tenacity. If they are equal in tenacity, they separate themselves in accordance with fungicidal value. Prévost (87) made the first prediction when he decided to use copper for wheat bunt because copper killed the spores. Those interested in wood and fabric preservation have developed in the laboratory many new fungicides such as the nitrated and chlorinated phenols. Among recent fungicides for farmers that were developed in the laboratory are tetrachloroquinone (Spergon), 2-3 dichloro 1-4 naphthoquinone, disodium ethylene bisdithiocarbamate (Dithane), lauryl iso-quinolinum bromide (Q15) and phenyl mercury triethanolamine lactate (Puratized N5x).

Before many years have passed the number of new materials will be so large and so specific in their action that Bordeaux mixture and elemental sulfurs will be turned out on pasture to spend their last years in leisure as a reward for a good job well done.

SUMMARY

The field for fungicides is expanding very rapidly now with the demands for protecting military materiel from decay, and with the rise of organic compounds in the field of agriculture. Such an expanding subject demands accelerated techniques for appraisal of new developments.

With a few important exceptions in the fields of fumigation and therapy, fungicides find their major usefulness as protectants. As such they must be applied to the object to be protected before penetration has occurred. Application can be made before inoculation, as wood preservation or control of apple scab, or application can be made after inoculation but before penetration, as in the control of peach leaf curl or wheat bunt.

Protective value of a fungicide is supported on two legs—dosage and fungicidal value. Dosage concerns itself with the quantitative factors in the action, deposition, adherence of the

materal, retention by the treated surface, and tenacity or resist-
ance to weathering. The dosage factors are, in modern parlance,
the factors of logistics—to have the proper amount of material
at the proper place at the proper time.

Fungicidal value concerns itself with quality factors—availa-
bility and inherent toxicity. It is not proper to speak of the
fungicidal value of a material in the control of apple scab. That
is its protective value. Its fungicidal value is its ability to kill
the spores. Formaldehyde would have high fungicidal value, low
protective value. Availability is concerned with the making of
a fungicide out of an insoluble residue. It is concerned with the
speed of solubility. A material with small particles is more
"available" than one of the same kind with large particles.

Inherent toxicity is the ability of the toxicant once made
available to kill the fungus concerned. Copper chloride has a
greater inherent toxicity than zinc chloride, but copper oxide
has a smaller fungicidal value than zinc chloride because copper
oxide is not so available as zinc chloride.

If these factors in fungicidal action are to be appraised, tech-
niques will have to be designed. Far and away the most satis-
factory technique from most points of view is the spore-germina-
tion technique. It is rapid and it can be operated as a reasonably
pure chemical system without foreign contamination, as is in-
volved in agar. If desired, contaminations can be introduced.
The method is precise within experiments and reasonably re-
producible.

In general, the assay procedure for protectants with the spore-
germination technique is to spray a given surface, usually a
microscope slide coated with cellulose nitrate. Precautions must
be taken against drifting of the spray stream in the air, against
evaporation in transit and against differences in surface-tension
because these may affect the size of the droplet emitted by the
sprayer, the speed of travel and the coverage of the surface.

Tenacity or resistance to weathering can be assayed by giving
the sprayed surface a predetermined washing.

Although it is desirable to use the fungus under consideration,
it is usually necessary to use some other that sporulates and
handles readily, such as *Sclerotinia fructicola* or *Macrosporium
sarcinaeforme*. Precautions must be taken to take standard-aged

spores, to maintain uniformly dense spore suspensions, to regulate the diameter of spread of the spore drops, to incubate at optimum temperature for the fungus, and to permit enough time for all spores to germinate.

It is desirable to use a series of doses for each material and to determine the percentage of mortality (usually from 100 spores) for each dosage. From such data a dosage-response curve can be plotted. It will usually plot to a straight line on logarithmic-probability paper. If it does not, all elements of technique, such as volatility, diffusion and coverage, should be scrutinized before deciding that the line is really other than straight.

Such a line provides LD values and slope, that is, the lethal dose for any given level of response, such as LD 50 or LD 90, and the angle of the line. Without becoming involved in statistics unduly, it may be said that LD values measure the dose factors of quantity of deposit and also availability since availability is really a dose factor. LD values, therefore, are important in quality control of a fungicide. They show differences in particle size, *etc.*

Slope is a measure of inherent toxicity. If compounds act differently, they will show different slopes. Slope, however, may measure coverage also.

Poisoning of the food offered to a fungus is an old method of assay, and it has its advocates. Its major advantage seems to be that it permits use of the organisms of primary concern. Other than that, it is slow. It is incapable of appraising tenacity.

Some experimenters hold vigorously for using the object to be protected, such as real paint films, fabrics, wood or the growing plant. To use these slows the technique considerably. The chief advantage of using them is that the action may be different from what it is on the microscope slide. There is some evidence that the action is different. As soon as the factor causing the difference is known, however, it can be placed under scrutiny and measured by the spore-germination technique.

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THE CONTROL OF FUNGI IN LUMBER DURING AIR–SEASONING

ARTHUR F. VERRALL[1]

INTRODUCTION

Freshly sawed lumber placed in air-seasoning piles for drying is subject to the attacks of stain, mold and decay fungi until the moisture content of the wood is below the fiber-saturation point. At this point the free water in the cell lumina is gone and nearly all fungus activity ceases;[2] when thoroughly air-dry, *i.e.*, with less than 20% moisture, wood is practically immune to fungus attack so long as it remains dry. When lumber production consisted largely of old-growth virgin wood, which contained little sapwood, potential degrade of air-seasoning lumber because of fungus attack was considerably less than now. Much of the present cut is sapwood, which is particularly subject to important fungus staining and usually is more susceptible to molding and decay than is heartwood. Undoubtedly this increased proportion of sapwood is one of the main reasons why degrade caused by fungi became alarming in the 1920's, particularly in the South where much of the lumber cut is air-seasoned, and led to the studies during the early 1930's that improved control methods.

A summary of the information then available on the control of fungi in air-seasoning lumber was prepared for the National Committee on Wood Utilization in 1929 (37). Recommended control methods consisted of the use of solutions of sodium carbonates as dips, and practices to prevent log infections and to promote rapid drying in the seasoning yard. Under commercial conditions the soda dips have given uncertain protection to pine and little or no protection to hardwood lumber, and such practices as pre-steaming and end-racking of lumber, designed to give rapid initial surface drying, cannot be depended on to give adequate control under many conditions (47).

[1] Division of Forest Pathology, Bureau of Plant Industry, Soils and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.
[2] Although the mass of evidence is that decay and stain fungi cannot develop in wood materially below the fiber-saturation point, there is some observational evidence that molds may be able to develop at slightly lower moisture contents, but proof is lacking.

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Fungi causing degrade in green lumber are conveniently divided into three groups: mold, stain, decay fungi. The mold and stain fungi are mainly Ascomycetes and Fungi Imperfecti that live chiefly on cell contents. The stainers, with dark hyphae, discolor the wood; the typical molds, with hyaline hyphae for the most part, cause only surface discolorations by colored spore masses. The decay fungi are mainly Hymenomycetes and attack the cell walls. These groups are purely arbitrary and may overlap, as do some of the decay fungi which cause characteristic discoloration in the incipient stages of decay; in general, however, the important fungus species are distinguishable on the group characteristics mentioned.

These stain, mold and decay fungi exhibit wide ranges in growth rates (9, 20, 21, 28, 29, 38, 42, 44, 48, 61, 64, 77, 79) and in tolerance to chemicals (7, 8, 11, 32, 36, 40, 55, 67, 68, 74). For these reasons many of the practical problems of fungus deterioration cannot be easily studied to the best advantage in experiments with individual fungi under controlled conditions, and the main problems in the control of fungi in seasoning lumber have been studied in the field. Often these field studies have been difficult to interpret in terms of definite environmental conditions, but, on the other hand, they have yielded enormous returns in terms of commercial usefulness for the time and expense involved.

GENERAL ASPECTS OF CHEMICAL CONTROL

With certain woods, particularly those with high percentages of heartwood, and with most woods during the drier or colder seasons in some regions, provisions for rapid drying may be sufficient to secure bright air-seasoned lumber without chemical treatment (68). However, early in the work on fungus control in air-seasoning lumber it was apparent that chemical treatments must play a decisive rôle in fungus control during all seasons in such warm humid regions as the lower Mississippi Valley and in most regions during certain seasons. Up to 1930 no generally satisfactory chemicals had been found (68). The effective chemical control now in general use resulted from extensive cooperative studies by the chemical and lumber industries and the Division of Forest Pathology of the U. S. Department of Agriculture. The results of these studies appeared periodically in trade journals and were summarized in 1940 (68). Among the large number of

chemicals or combinations tested on air-seasoning lumber two groups were of outstanding success: (*a*) organic mercurials, especially ethyl mercuric phosphate and ethyl mercuric chloride at about 0.015%; and (*b*) certain chlorinated phenols at concentrations of 0.85% to 0.96%, especially sodium pentachlorphenate, sodium tetrachlorphenate and a 1–1 mixture of sodium tetrachlorphenate and sodium 2-chlor-o-phenylphenate. These chemicals best combined the qualities of effectiveness, cheapness, ease of handling and other qualities suiting them to wide commercial use. In addition to these, two previously tested chemicals, soda at about 8% and borax in saturated solution (68, 37), are still occasionally recommended for commercial use, even though the first of these has not given consistently good fungus control under severe seasoning conditions and the latter has been found consistently good only on gum (68). Satisfactory control has been secured with several other organic and inorganic materials, but various factors, mainly coloring of the wood, cost and corrosiveness to metal, have prevented their use (55, 68).

Commercially, fungicides are applied mainly by passing the lumber through a water solution of the chemical, although they are sometimes applied as sprays (68) or even by brushing. These methods afford only a surface protection during the air-seasoning period but little or no long-time prevention of decay during subsequent use.

Most of the tests on chemical control dealt primarily with the exclusion of stain fungi, although mold and decay fungi were given some consideration. As will be pointed out later, some of the chemicals recommended for general use against stains are not particularly effective against molds under severe conditions. The effectiveness of chemical dips in controlling decay fungi is more difficult to determine by visual means, but from observations on field tests (68) and laboratory toxicity tests (32) it appears that wood-destroying fungi can be adequately controlled during the seasoning period by most of the chemical dips recommended for commercial use against stain fungi. Further evidence on decay control has been obtained by tests (88) in which bright chemically treated lumber had higher strength values than stained untreated lumber, the loss in strength in the latter presumably being due partly or

mainly to decay fungi present, since stain fungi alone ordinarily do not cause material weakening of wood (68).[3]

Many of the chemicals used as general fungicides on plants or in the soil have proved ineffective in preventing fungus attack on lumber, at least in concentrations within competitive cost limits: lime-sulphur (34, 49), copper carbonate (49), Bordeaux mixture (49), colloidal sulphur (49), formaldehyde (36, 49), tetrachloro-para-benzoquinone (55, 83). In recent years there have been developed a number of surface-active detergents of high bactericidal value (14), some of which are known to be toxic to fungi (35). Among these detergents alkyl dimethyl benzyl ammonium chlorides, para-tertiary-octyl-phenyl-diethoxy-dimethyl-benzyl ammonium chloride monohydrate, and three proprietary cationic detergent disinfectants have been tested for the prevention of fungus development on seasoning lumber and found to have little or no value for this purpose (55, 83). Also, the dithiocarbamates have proved ineffective (55, 83).

THE INFLUENCE OF FUNGUS FLORAS

Because various fungi or groups of fungi show differences in their tolerance to toxic chemicals, the differences in fungus floras attacking various lumber species in various localities have a bearing on chemical control. A few species of stain fungi are widely distributed, as *Ceratostomella pini,* which has been reported from the United States (62), Japan (58), Russia (77), Great Britain (51), Germany (56) and Scandinavia (42), and *C. ips* reported from the United States (62), Japan (57) and Italy (31). However, the important stain floras vary with widely separated geographical localities (15, 31, 42, 51, 56, 76, 77, 79), with wood species (15, 77, 79), and to a less extent with season (79). Wherever extensive studies have been made, important staining has been found to be caused by several species. For a given wood species, differences in stain floras with locality have been observed in at least one instance. On *Pinus sylvestris* in Russia, *Ceratostomella coerulea* and *Endoconidiophora coerulescens* are said to be common in the Archangel Province but absent in the Leningrad

[3] Stain does cause some reduction in toughness (68), but this is not significant except for specialized uses. Recent studies (24), however, show that *Diplodia natalensis* causes more strength loss than the more common *Ceratostomella* spp.

Province (77). Such differences have not been observed in widely
separated localities in southern United States (79), although during
limited intervals many of the important species may be scarce, or
less important species may be abnormally abundant. Therefore,
if observations are for limited periods apparently important differ-
ences in stain floras on a given wood species can be found in
different localities. The species of mold and decay fungi attacking
seasoning lumber have not been adequately studied so that flora
differences by localities or hosts in relation to control are uncertain,
but it is known that there are some differences with wood species
and localities.

In general, the geographical range of fungi is determined pri-
marily by that of hosts or substrata, although in some instances
other factors may be involved (6). With wood-inhabiting fungi,
variations among species in cardinal temperatures for growth are
sufficient to restrict the distribution, particularly locally, to certain
use-conditions (9, 38, 64). The restriction of fungi by high tem-
peratures should be less apparent in wood during the early periods
of air-seasoning, since temperatures inside seasoning piles remain
favorable for the development for most wood-inhabiting fungi
when outside temperatures are near or above the maximum for
growth of many (48). This cooling effect of evaporation is great-
est during the early seasoning period, *i.e.*, the period during which
fungus deterioration is greatest. During periods when the air
temperatures are in the lower range for fungus activity it is con-
ceivable that the cooling effect of evaporation might reduce the
temperatures inside piles below the minimum required for certain
fungi.

Physiologic specialization within the important lumber-inhabit-
ing fungus species has not been extensively studied, and many re-
ports of this phenomenon are based on single tests that have not
been verified. Strains of *Ceratostomella pilifera* differing in tem-
perature relations (48) and reactions to chemicals (32), strains
of *C. coerulea* differing in temperature relations (48), and strains
of *C. pini* differing in cultural characteristics (62) and tolerance to
chemicals (32) have been reported among wood-staining species.
The differences in hosts for European (42) and American (15)
isolates of *Endoconidiophora coerulescens* can now be ignored,
since the American isolates have been shown to comprise a distinct

species (16). Among molds from wood, forms of *Trichoderma lignorum* with widely different cellulose-dissolving ability have been found (71). Several species of wood-destroying fungi are known with forms varying in temperature relations (38), wood-destroying abilities (4, 69, 78) and pH relations (50). Judging from the common occurrence of physiologic races within fungus species in many taxonomic groups (72), it would seem likely that the knowledge of this phenomenon among wood-staining, -molding, and -destroying species is very incomplete.

The important fungi staining pine and hardwoods in southern United States are about the same on wood treated with ethyl mercuric chloride, or a mixture of sodium tetrachlorphenate and sodium 2-chlor-o-phenylphenate as on untreated wood (81). However, chemical treatments are not equally effective against all fungi. Organic mercurials permit the development of blue mold (*Penicillium*) (67) which is seldom bothersome on untreated lumber or that treated with other chemicals.[4] Treatments containing fluorides, as magnesium, sodium and zinc silicofluoride, are usually conducive to a heavy development of *Trichoderma* (55, 67). Borax appears particularly effective against wood-destroyers but oftentimes permits objectionable development of an unidentified surface-stainer (55, 68). Other cases include the tolerance of *Hormodendrum* to creosote (11), *Aspergillus* to copper sulphate (36), and a wide variety of fungi to arsenicals (7, 40, 74). The tolerance of *Hormodendrum resinae* to creosote and coal tar is particularly interesting, since this fungus not only tolerates higher concentrations than any other known fungus but apparently can grow and reproduce with no other source of nutrients than these materials. Yet this fungus is no more resistant to inorganic toxicants than a number of other fungi.

These biological differences in conjunction with varying environmental factors have made it advisable to test the effectiveness of different chemicals for each region. The organic mercurials have proved generally effective against stain fungi on all wood species wherever tested: United States (68), Finland (46, 65, 66), Great

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[4] The reviewer has extensive unpublished data showing that the amount of *Penicillium* on pine sapwood treated with ethyl mercuric phosphate increases markedly, under poor drying conditions, as the concentration of the mercurial is increased from none through various concentrations up to that used commercially for stain control.

Britain (23), Malaya (53, 75), Australia (13), Philippines (7), Canada (18, 26). Likewise, sodium pentachlorphenate has proved satisfactory on all species where it has been tested: India (2), Finland (59), Gold Coast (52), Nigeria (86), United States (55, 68, 83). A 1–1 mixture of sodium tetrachlorphenate and sodium 2-chlor-o-phenylphenate has proved satisfactory in Canada (18), Great Britain (23), Rhodesia (25) and Finland (65). Other chemicals are of more limited usefulness. Sodium tetrachlorphenate alone or in combination with borax has proved satisfactory on southern hardwoods, but not on southern pines (55, 68), but is effective on conifers in the Pacific Northwest (18). Borax is effective or fairly so on *Podocarpus* in New Zealand (17), on *Dyera* in Malaya (75), and on American hardwoods but not on conifers (68).

<center>CHEMICAL MIXTURES</center>

Because fungi or groups of fungi differ in tolerance to some chemicals and because of the multiplicity of species of stain, mold and decay fungi that develop on seasoning lumber, treatments by single chemicals must be at relatively high concentrations in order to exclude all fungi. It is logical that the ideal treatment be a mixture of two or more chemicals from widely varying groups not only to allow protection against a wider range of organisms but also to reduce the necessary toxic concentration, thus reducing the cost and the dangers of injury to workmen. For example, ethyl mercuric phosphate is ordinarily used at the rate of 0.06 pound to 50 gallons of water, sodium pentachlorphenate at 3.5 pounds to 50 gallons, and borax at 16 pounds to 50 gallons, or as much of this as will dissolve at the prevailing temperature. If each of these concentrations is considered 100% in a scale of toxicant concentration, a mixture containing 0.008 pound of ethyl mercuric phosphate, $\frac{1}{2}$ pound of sodium pentachlorphenate and six pounds of borax to 50 gallons of water would contain a toxicant concentration of about 65%. Yet this triplex mixture has proved about as effective as any of its components alone at full concentrations (55, 83). The mixture of sodium pentachlorphenate two pounds and borax three pounds per 50 gallons also has proved effective experimentally (55, 83) and is in commercial use. The advantages of such a mixture as sodium tetrachlorphenate and sodium 2-chlor-o-phenylphenate, which is in wide commercial use,

would not seem great, since the components are closely related and both cause dermatitis. The development of mixtures based on sound experimental work on toxicity seems to offer considerable promise of improving methods of chemical control of fungi. Of the mixtures so far tested the most promising for use on green lumber (55, 65) seem to be those containing borax in duplex or triplex mixtures with certain sodium chlorphenates and organic mercurials, and duplex mixtures of chlorphenates and organic mercurials. However, the most advantageous proportions of the various components have not been determined. Also, other factors need attention before low concentration mixtures can be recommended for commercial use. Does the borax maintain sufficient alkalinity of the treated wood surfaces to prevent conversion of the soluble phenate to the insoluble phenol, thus increasing the chance of loss by rain wash? Solutions of the ethyl mercuries are known to lose toxicity with use (26), probably through selective adsorption of the mercury by wood fibers. Consequently, this may seriously reduce the toxicity of solutions containing a low initial mercury content.

No studies on possible synergistic effects on wood-inhabiting fungi have been encountered. Such effects might occur but could not be detected in field studies in which wood is attacked by many species of fungi. It is more reasonable to assume that the effectiveness of mixtures on air-seasoning lumber is due more to the various components being effective against different fungus species than to synergism.

EFFECT OF THE SEASON OF TIMBER FELLING ON RESISTANCE TO FUNGUS ATTACK

It has long been believed by wood users that wood felled in the winter is more resistant to fungus attack than that felled during the growing season. Timber cut, milled and placed in air-seasoning piles during the winter usually has a lower surface moisture content at the advent of weather warm enough for rapid fungus infection. Limited and rather inconclusive experimental data from Sweden (42) indicate no consistent differences in susceptibility of winter- and spring-felled spruce and pine to blue-stain fungi under laboratory conditions. Field tests show that severe lumber staining and molding can occur in southern United States

during both winter and summer, weather conditions permitting (10, 55, 83).

The only extensive researches on the effect of season of felling on the susceptibility of wood to fungus activity are those of Gäumann (27, 28) who, from laboratory and field tests in Switzerland, concluded that spruce and fir felled during the period of active stem growth was at least twice as susceptible to decay as that cut during the winter. The differences, however, were of little practical significance in the wood that had been fully seasoned. With beech the seasonal differences found were not of practical magnitude. Although these data are outwardly convincing and have been generally accepted by reviewers, they should be confirmed by further tests before being accepted. Doubt is raised because the seasonal differences in susceptibility to decay were reported for the relatively inert heartwood as well as the sapwood, and the sharp rise in decay susceptibility started with the felling at the middle of February, before one would expect much growth activity. Only under the most completely controlled conditions is it possible to get reliable comparative data, even when all samples are tested simultaneously. With tests started during different seasons, and each extending over a period of several months, it would be extremely difficult to maintain uniform conditions, particularly moisture content of the test sample. Certainly Gäumann's researches bring out interesting possibilities, and it would seem distinctly worth while that further work on the effect of time of felling on durability be done, particularly with some of the more decay-resistant woods as white oak and bald cypress.

NECESSARY ADJUNCTS TO CHEMICAL CONTROL

There is ample evidence based on experimentation and observations under commercial conditions that the use of chemicals on green lumber in itself will not always insure adequate control of fungi during the subsequent air-seasoning period. The short period dips or sprays used in treating green lumber are relatively superficial and cannot be expected to kill previous infections deep in the wood or to be effective over protracted periods. Therefore, chemical treatments must be supplemented by handling methods that permit treatment before infections become deep-seated and that permit rapid drying after the chemical is applied.

Quick Utilization of Logs. Deep-seated infections of logs prior to sawing into lumber raise one of the most serious problems in the control of fungi in seasoning lumber (47, 80, 82). In cold weather or in logs that are sawed soon after cutting, log infections are seldom bothersome. The length of time fresh logs can be safely stored out of water varies so much with locality, season, wood species and other conditions that no general recommendations can be made. Spraying logs with fungicides or the use of appropriate end-coatings are effective means of preventing fungus infections so long as insect attack is not prevalent (41, 68, 73, 91). The fungicides in use show no deterrent effect on bark and wood-boring beetles (52, 68, 82) that are effective agents in inoculating through the protective chemical shell. The constant association of certain fungi, including staining species of *Ceratostomella,* and bark and ambrosia beetles is fully established (19, 43, 62, 63, 64, 82, 84, 89, 90). Inoculations by beetles often result in infection of standing trees and logs. From the point of view of log protection there is a need for cheaper methods of beetle control. Recent studies (12) show that chemical control of beetles is possible, but the really effective chemicals are rather costly. Storage of logs in water is also an effective method of preventing both fungus and beetle damage (5, 42) provided the entire log is kept wet. In southern United States, at least, water storage is much less common than in the past because the present logs, with a greater sapwood content, are higher in specific gravity and sink so soon that raising them increases handling costs excessively.

Although one of the main purposes of water storage is to maintain during the storage period conditions unfavorable for fungus and insect development, there is also the possibility that water storage may have an influence on the susceptibility of wood to fungus attack after sawing into lumber. The available data are not conclusive, but they indicate that the effect of water storage on subsequent fungus activity would vary with fungus and wood species. Preliminary studies indicate that prolonged water storage of coniferous logs reduces the susceptibility of lumber cut from them to attack by some stain fungi (1, 42) but not by others (42). Water-stored coniferous logs seem more susceptible to attack by the mold *Trichoderma* (22), which may render the wood less suitable for other fungi, possibly because of toxins produced.

Water storage also tends to deplete stored carbohydrates and thus reduces attack by some beetles (60, 87) and perhaps certain fungi (87). In contrast, should the wood be one naturally resistant to fungi because of water-soluble toxic extractives (33), water storage might sufficiently reduce these to render the wood more susceptible to attack (70); should the extractives not be especially water-soluble, water storage for usual periods would not be expected to decrease durability greatly (54). Furthermore, storage in sea water or in pond water loaded with extractives from previous lots of logs might give results different from fresh water.

In northern European literature there are records of tests of novel methods of treating or storing logs to prevent fungus attack: natural resin impregnation (39), continuous water sprays (45), and covering log piles with a thick layer of coniferous boughs (42). For economic or climatic reasons these would not seem feasible under many American conditions. Also, it seems doubtful that some of the methods described are in fact effective, since the evidence is conflicting (42, 77).

Minimum Delay Between Milling and Chemical Treatment. Lumber leaving a sawmill is likely to be heavily inoculated with fungi (82), and chemical treatment must be done before these inoculations develop into deep-seated infections. The rate of growth of fungi, particularly the rate at which they penetrate wood, has an important bearing on this problem. Different important stain fungi have widely varying growth rates, ranging from about 2 to 20 mm. in radius per day on agar media at 25° to 30° C. (42, 48, 79), which is near the optimum temperature for many stain and decay fungi. The rate at which *Ceratostomella pilifera* penetrates pine wood tangentially, radially and longitudinally, approximates the ratio 1:2:9, the longitudinal penetration being about equal to the radial growth on agar under favorable growth conditions (48). The same fungus under favorable laboratory conditions penetrated pine wood sufficiently far in 48 hours to escape killing by a 10-second dip in ethyl mercuric chloride; boards dipped 24 and 12 hours after inoculation developed but slight interior stain. European studies on penetration of stain fungi (42), although not easily interpreted in relation to chemical control, indicate that chemical treatments 24 to 48 hours after sawing would kill most stain-fungus infections. Assuming that the corre-

lation between growth on agar and penetration in wood found for
C. pilifera (48) holds for other stain fungi, some of the faster
growing species, as *Diplodia natalensis,* with a growth rate on agar
of four to five times that for *C. pilifera* (79), might under ideal
conditions penetrate wood sufficiently far in 12 hours to escape
killing by subsequent chemical treatment. However, field tests
(68) show that under usual commercial conditions satisfactory
control can be secured if lumber is dipped within 24 hours from
the saw, provided there are no deep-seated log infections prior to
milling. During cool weather longer periods of delay probably
would be safe.

Although this recommendation is made for the control of stain
fungi, it should be applicable to mold and decay fungi also. Most
molds cause degrade mainly by the presence of colored spores on
the wood surface (68). Those molds studied, however, have been
found to penetrate wood (30, 67, 71, 85). *Trichoderma lignorum*
appears to penetrate pine wood more slowly than does *C. pilifera*
(68, 71). Also, molds are of less economic importance, the visible
evidence of them being removed by the usual surface planing or
by a mechanical brush treatment (3). No information is avail-
able on the rate of penetration of decay fungi in seasoning lumber.
On agar a large number of decay fungi grow at rates (9, 38)
about equal to the stainers of intermediate growth rate (42, 79),
and with none approaching the growth rates of the faster growing
stainers.

Reducing Bulk-Piling Periods to a Minimum. In lumber han-
dling practices it is sometimes necessary or desirable to put treated
lumber in solid or bulk piles for varying periods before it is placed
in regular, ventilated air-seasoning piles. The effectiveness of the
commercial fungus-control chemicals in protecting lumber during
bulking periods beyond seven weeks is doubtful under the severe
conditions of southern United States (68), but much longer bulk-
ing periods seem safe in the Pacific Northwest (18), particularly
if higher than normal concentrations of the chemical are used.
However, there is only fragmentary evidence on how much fungus
activity will subsequently occur in air-seasoning piles after the
bulking period (68). Further information is needed before posi-
tive conclusions can be drawn.

Good Air-Drying Conditions. To insure good stain control by

chemical treatments it is necessary to have rapid drying provided
in the seasoning yard by good soil drainage, weed control, and
arrangement and construction of piles to facilitate air movement;
and to protect the treated lumber from rain wash both before and
after piling (68). The experimental evidence for these statements
is rather meager, but from theoretical considerations, experience
with chemicals under severe conditions in small scale tests (55, 68,
83), and extensive observations in commercial yards there is little
doubt that provisions for rapid drying are necessary.

Avoidance of accumulations of wood refuse in and around sea-
soning yards is usually listed as a desirable practice in stain control
(37, 68, 80), although there is no direct evidence for this. Gen-
eral observations over a period of years indicate that the deleterious
effect of such accumulations would, under most conditions, be
limited to the effect of obstructing air movements, and that an
abundance of inoculum is present even in those yards with the
least wood refuse. There is some experimental evidence sup-
porting this (82).

CONCLUSIONS AND SUMMARY

The control of mold, stain and decay fungi in lumber during air
seasoning has become progressively more important as the amount
of the more fungus-susceptible sapwood increases with the passing
of virgin timber stands and as consumer demands become more
critical. This control is best accomplished by the use of chemical
dips in conjunction with practices that prevent deep-seated log
infections, that permit quick chemical treatment after sawing into
lumber and that promote rapid drying in the seasoning yard. It
is estimated that under peacetime conditions as much as $3\frac{1}{2}$ billion
board feet of lumber annually are dipped in chemicals for the
prevention of fungus deterioration in seasoning yards.[5]

Some of the chemicals used on green lumber have been found
satisfactory on all species of wood and in all regions where tested;
others are satisfactory only on certain woods, particularly in certain
regions. Some are not effective against molds, although highly
effective against stain and decay fungi. Because green lumber is
attacked by large numbers of fungi with varying tolerances toward
certain chemicals, single-chemical treatments are usually applied at

[5] Based on informal reports of the sale of anti-stain chemicals.

relatively high concentrations to afford an adequate factor of safety. The most recent tests on the control of fungi in green lumber show that mixtures of chemicals offer promise of improving control by reducing the total toxic concentration needed, thus reducing the cost and dangers of injury to workmen. This is presumably due mainly to giving protection against a wider range of fungi. More work is needed on the most advantageous mixtures; the effect on the solution of selective adsorption by the wood, particularly when the chemicals are used in dilute concentrations; and the effect of rainwash on mixtures containing borax.

Of the factors influencing the effectiveness of chemicals on green lumber one of the most bothersome is that of deep-seated infections in logs prior to sawing into lumber. Much of this infection could be prevented by proper integration of woods and milling operations to prevent long-time log storage. Since this is not always feasible there is a need for more effective chemicals for use on logs. Effective and cheap fungicides are available, but they do not repel bark and ambrosia beetles that penetrate the logs and inoculate the wood inside the protective chemical shell. The development of effective and cheap beetle repellents is needed.

In seasoning lumber the actual mechanics of fungus control are better known than many of the biological factors involved. The mills that follow recommended practices generally get bright lumber at moderate cost. Yet the reason why certain chemicals (as borax) are effective only on hardwoods or others (as sodium tetrachlorphenate) on hardwoods only in one region, but on both hardwoods and conifers in another region, is not certain. Perhaps it is merely a matter of differences in fungus floras, although chemical or physical chemical reactions between the fungicide and the different woods may be a factor. More information is needed on fungus floras on different woods in different regions, particularly those associated with failures of chemicals. This information would afford a better basis for devising chemical mixtures.

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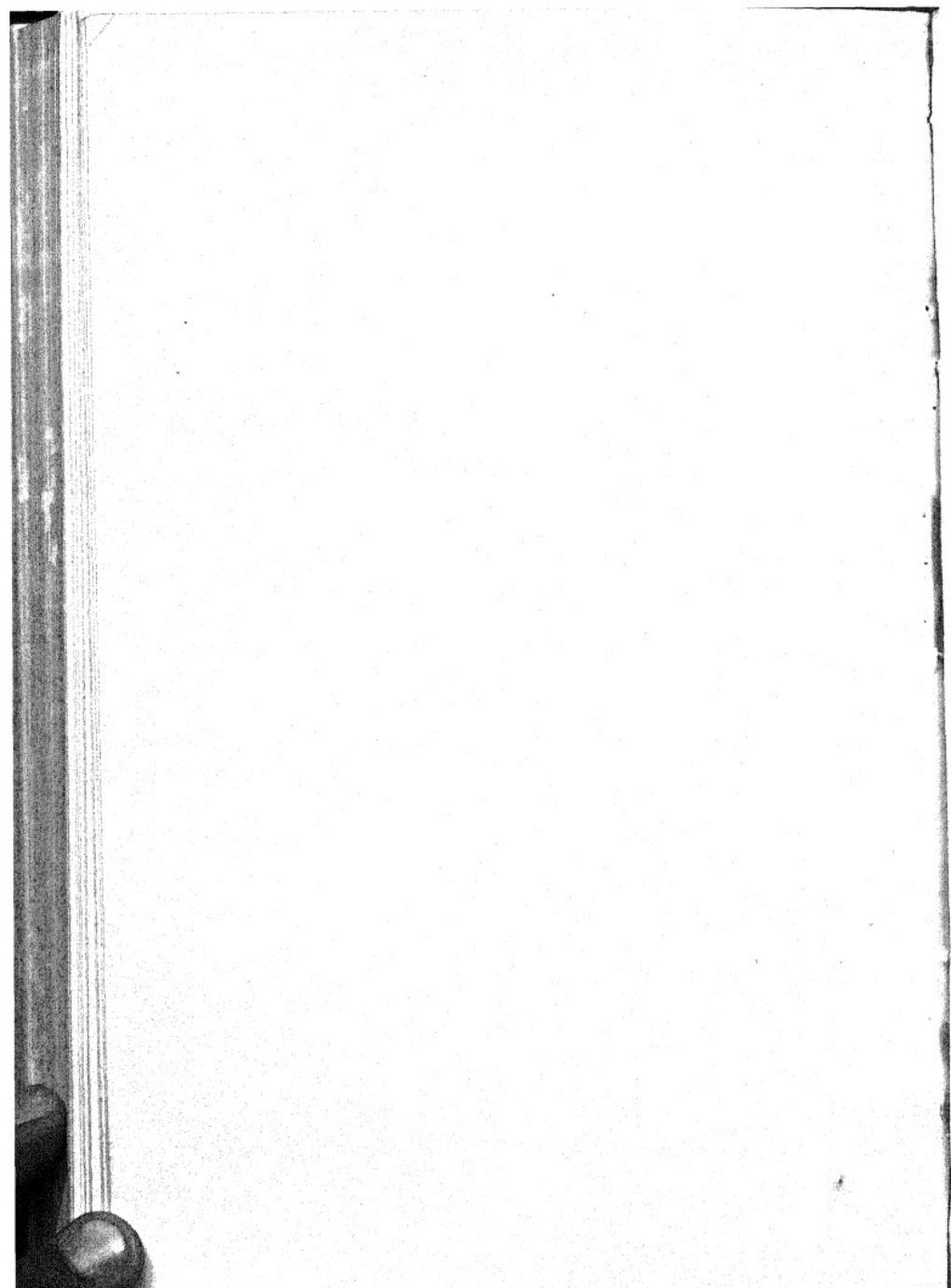
A REVIEW OF LITERATURE ON *TARAXACUM KOK-SAGHYZ* ROD[1]

G. KROTKOV
Queen's University, Kingston, Ontario

INTRODUCTION

The recent rubber shortage in North America has induced considerable interest in every species of plant that might possibly serve as a source of natural rubber. Martin (120) has recently given a brief description of a number of such plants.

The Russian conclusion, that for cultivation outside the tropics kok-saghyz is one of the most promising, has led to the importation of this species into America, and for the last two and a half years it has been the subject of investigations in the United States and Canada.

This period of study is as yet too short to have resulted in many English publications, and the bulk of literature on kok-saghyz remains in Russian. Unfortunately this renders it almost inaccessible to the majority of English-speaking workers. To overcome this situation the National Research Council of Canada undertook a survey of the Russian literature on rubber-bearing plants in general and on kok-saghyz in particular. The survey has been carried out by the present author and this review is one of its results.

Owing to the scattered distribution of Russian publications it would be impossible to bring together just now the entire literature on kok-saghyz. Accordingly the scope of this review has been restricted to a survey of those papers that are to be found in certain American and Canadian Libraries and hence are most readily available to the North American investigator.

DISCOVERY AND BIOLOGY OF KOK-SAGHYZ

It was not until 1926 that a campaign was begun in the Russian press, urging the necessity of re-examination of the Russian native

[1] Issued as N.R.C. No. 1262.

flora for new rubber-bearing plants which were growing under the climatic conditions of the U.S.S.R. (35). In 1928 a species of *Chondrilla* on the shores of the Caspian Sea was the first native rubber-bearing plant to be found. In a search for more extensive wild stands of it, a second native rubber-bearing plant—tau-saghyz—a species of *Scorzonera,* was discovered in 1929. Analysis of its dried roots revealed that they contained close to 40% of rubber. From these two examples it became clear that the Russian native flora contained some plants with considerable rubber in them, and that such plants were not known to Russian botanists. Early in 1931 it was decided to re-examine the Russian native flora, and a period of two years was given for this purpose. The actual execution of this re-examination was entrusted to Bosse and Prilutskaia, whose paper (35) represents their report.

In 1931 an expedition sent to Kazakhstan and headed by L. E. Rodin returned in the fall with the roots of a native plant called "kok-saghyz". In local language kok-saghyz meant "green chew", since the plant is used by the local population as a masticatory. The roots were planted in a greenhouse of the Botanical Institute in Leningrad where they produced leaves and flowers, and it was from these specimens that the first botanical description was given under the name of *Taraxacum kok-saghyz* Rodin (118).

In 1932 a special expedition was sent to Kazakhstan to study kok-saghyz in its natural habitat and to report on the feasibility of its introduction into cultivation. The report subsequently was given by S. Lipschitz (109). According to it, the native habitat of kok-saghyz is in the valleys of the Tien Shan mountains, between 79° and 80°30′ east longitude, 42°20′ and 43°20′ north latitude. The elevation of this region varies between 1,800 and 2,000 meters above sea level and its climate is continental. While the soil conditions in these valleys vary considerably, kok-saghyz prefers to grow on soils which have from average to low salt content, large amounts of humus and a high degree of moisture. Kok-saghyz plants often invade cultivated fields where they thrive and grow luxuriantly.

This expedition reported kok-saghyz to be a very promising plant for rubber production, and recommended its introduction into cultivation. It was also suggested that until cultivated plantations of it become established, its wild stands should be looked upon as

temporary sources of seeds. Collection of seeds from these wild stands has been described by two authors (37, 89). According to the first, the total area of kok-saghyz stands is close to 2,000 hectares (hectare = 2.47 acres). These are distributed in the three valleys of the Tien Shan mountains and are scattered among the local meadows in areas of 0.5 to 175 hectares. According to the second author, in order to insure that collected kok-saghyz seeds were not contaminated by those of other dandelions, they were accepted from the local population only during the season when seeds were produced on the kok-saghyz plants. As a further precaution, only whole heads of plants, never seeds alone, were accepted. The presence of "horns" on the involucral bracts of kok-saghyz heads was the main characteristic used for the separation of these heads from those of other dandelions. It was later found, however, that in spite of such precautions, the seed material collected from the wild stands contained some admixture of other dandelions. According to Lipschitz (109), in 1932 this admixture was about 5.8%. Koroleva (94), however, reported that in one case seeds collected from a wild stand produced more than 50% of other dandelions than kok-saghyz. This worker described nine different species of *Taraxacum* which may be found growing together with kok-saghyz, and she gave the characteristics for their separation.

Taraxacum kok-saghyz Rod. is a perennial dandelion, and its general appearance is somewhat similar to that of the common dandelion. In its native habitat there have been found specimens with roots probably seven years old (109), while plants growing on a plantation for four years were also reported (94). Commercially, kok-saghyz is grown either as an annual or a biennial. It has either a tap or a branched root, and in its native habitat the average air-dry weight of a root is 0.8 to 1 gram with the rubber in it varying from 2.98% to 27.89% (109). The upper end of the root, known as the crown, bears either one or several rosettes of leaves. Each rosette has a diameter of 15 to 40 cm. and consists of 20 to 50 leaves which are more or less pressed against the ground. Leaves may vary in shape from lanceolate and obovate with practically entire margin, to pinnately lobed and even divided.

Later in the season each plant may produce up to 96 peduncles, each of which bears a head. Each head contains 25 to 50 flowers, protected by the involucral bracts two or three layers thick. Fruits

are small achenes, and about 3,000 of them weigh one gram.   As is true of other dandelions, they are disseminated by wind.

The main distinguishing characteristics of kok-saghyz which enable one to separate it from other dandelions are (94, 109):

(*a*) Threads of rubber between the two halves of a severed air-dry root are visible.

(*b*) Leaves are fleshy, without small teeth on their margins, and are bluish with a glossy surface.   The wide midrib is of light colour, while the side veins are poorly developed.

(*c*) Involucral bracts have small "horns" at their ends.

One of the striking characteristics of kok-saghyz is considerable polymorphism of its leaves.   In the same population one can find specimens with simple, pinnately lobed and pinnately divided leaves.   It has been thought by many students that such polymorphism indicates the existence of genetically different forms. Lipschitz (109), for instance, tried to determine whether there was any difference in the germination of seeds collected from plants with simple and with lobed leaves.   He found that the percentage of germination of seeds from these two forms was practically the same.   As early as 1934 it was reported (100) that the largest roots are possessed by plants with pinnately lobed and not with simple leaves.   On the strength of this observation it was suggested to select kok-saghyz plants with pinnately lobed leaves.

Lebedeva (104) investigated the effects of external conditions and of the ontogenetic stage of the plant itself on the morphological characteristics of its leaves.   She reported that with advanced ontogeny and with favorable external conditions there occurs on the same plant an increase in the percentage of its lobed and a decrease of simple leaves.   That external conditions may influence the forms of kok-saghyz leaves has also been reported by others (50, 134).

In the first year of growth kok-saghyz plants may begin to flower 60 to 70 days from the time of sowing, and the period of flowering may extend until late in autumn.   In the second year of growth flowering is much heavier, and on such a plantation it lasts one and a half to two months.   In a kok-saghyz head the florets are arranged in a series of concentric circles, and various stages in the opening of a head have been described by several workers (94, 137, 139).

Sexual reproduction is normal (158).   Cross-pollination is the

usual method, but self-pollination may occur occasionally. After pollination a pollen grain begins to germinate on the stigma within five minutes, and a pollen tube reaches the embryo sac within 15 to 20 minutes. Under favorable conditions fertilization has been observed to take place 40 minutes after pollination, and six days later the embryo was fully developed. The somatic number of chromosomes is 16, while the sperm and egg cells have eight. Pollination in kok-saghyz has been studied also by Ghilarov (60). These conclusions on the normal sexual reproduction (158) have been confirmed (209, 221).

In the first year of growth only a small percentage of kok-saghyz plants produce flowers. A comparison between flowering and non-flowering plants in the first year of their growth revealed that at the end of the growing season flowering plants attained a greater fresh weight than the non-flowering (124). While the percentage of rubber in such flowering plants was somewhat lower, due to their greater weight, the absolute amount of rubber in them was higher. Similar observations as to the difference between flowering and non-flowering specimens have been made on plants grown from stratified seeds (3). On the other hand, one can also find in the literature statements that from the commercial point of view plants not flowering the first year are more desirable (88, 95, 99).

Borthwick, Parker and Scully (32) reported that none of their photoperiods varying from 8 to 18 hours completely inhibited development of flower primordia on kok-saghyz into mature flowers, though on a 12-hour or longer photoperiod they flowered somewhat more freely. Siniavskii (191) reported the existence of one form which in the first year flowered only after treatment with low temperature, while the second form did not require low temperature treatment but flowered only on a long day.

Between the appearance of flower buds and production of seeds from them there is a period of about 16 to 18 days. Freshly gathered seeds usually have a low percentage of germination, but the percentage increases during subsequent storage. Thus Poptsov (167) cites a number of examples wherein the percentage of germination of seeds gathered early in July was between 20 and 25, while 12 days later it was doubled. According to the same author, the length of this period of post-harvest ripening varies from year to year and is affected by the conditions of storage. In order to elim-

inate such post-harvest seed dormancy, this author recommends stratification, and he carried out a number of experiments (166) to determine the best external conditions for such treatment.

The stratification technique recommended for general practice has been described by a number of authors (1, 10, 197, 212). Following their stratification and in order to facilitate subsequent sowing, seeds should be slightly dried until they run. It was reported, however, that when stratified seeds are dried even only for two to three hours, the percentage of their subsequent germination decreases (166). Levitt and Hamm (108) reported an increase in germination when the seeds were permitted to absorb water in amounts insufficient to induce germination, and then were dried.

Aksel'rod (3) found that treatment of kok-saghyz seeds before sowing not only resulted in more uniform germination but also had marked effects on the subsequent development of plants produced from such seeds. Such plants flowered ten to twelve days earlier, and a higher percentage of them flowered the first year. From his data this worker also concluded that seed treatment between 0° and +2° C. for 14 days is just as effective as for 25 days.

Sirotkin (193) has confirmed the earlier flowering of plants grown from seeds pre-treated at 0° C. This worker has also suggested that on the basis of their reaction toward low temperature treatment there are two different forms or races of kok-saghyz. One form is similar to a fall wheat which requires a considerable period of low temperature treatment before it is induced to produce flowers. The other form is like spring wheat in that it requires only a short low-temperature treatment. The beneficial effects of low-temperature treatment on subsequent flowering have also been reported by other workers (32, 99).

ANATOMY

Roots. Blokhintseva (21) studied changes in the anatomy of a kok-saghyz plant during the first year of its growth. A root of a one- or two-day old seedling was found to have a primary cortex six to eight cells thick, protected by epidermis and a well differentiated endodermis. The root is usually diarch, and it contains two groups of primary latex tubes. In a three-weeks old root the epidermal layer loses its root hairs and becomes cutinized. Vascular cambium actively produces secondary tissues with considerable excess of

phloem over xylem, and by that time primary cortex has been pushed to the periphery and is crushed. Secondary phloem has groups of secondary latex tubes arranged in two or three concentric circles, each group containing three or four latex tubes.

When flower buds appear the primary cortex has already been sloughed off and the secondary phloem is protected on the outside by a cork layer several cells thick. Secondary phloem contains seven or eight concentric rings of latex tubes arranged in groups, each group consisting of four to six latex tubes. At the time of fruit formation, secondary phloem contains nine to eleven concentric rings of latex tubes.

While all Russian investigators say that sieve tubes of kok-saghyz are very small and difficult to find, Artschwager and McGuire (19) investigated them more thoroughly. These two workers state that "although the sieve tubes of kok-saghyz are relatively narrow compared with those of members of the Cucurbitaceae, they form in their entirety a well developed system, capable of expediting translocation and making rapid growth possible".

In the second year of its growth, as is characteristic of all perennial dandelions (172), kok-saghyz sloughs off all secondary phloem produced the previous year. This process of sloughing off was first described by Rudenskaia (180). At the beginning of the second year of growth a cork cambial layer appears on the boundary between the region of the last year phloem and the new one. This cambium produces cork which separates from the rest of the root all the phloem produced the previous year. As a consequence all the separated cells die and are pushed to the outside.

These crushed tissues pushed to the outside contain all the rubber produced the previous year, and they form a compressed layer which in Russian literature is called a "glove". A series of diagrams to show the sloughing off of such a "glove" is given by several authors (125, 191). This "glove" is sloughed off the root shortly after the peak of seed production has been reached. In its native habitat a kok-saghyz root does not shed its "glove" but merely pushes it to its periphery. Thus Lipschitz (109) described an old root which had on its periphery five such "gloves". While some (180) think that a "glove" is sloughed off the root as a result of centrifugal pressure exerted by newly formed cells, others (122, 123, 127) believe that a "glove" is broken down by micro-organisms

in the soil. In support of their belief they cite the work of Kalinenko (75) who reported on the destruction of rubber by several micro-organisms.

Besides kok-saghyz plants of the usual anatomical characteristics described above, one occasionally finds specimens with a number of abnormalities. Thus roots with triarch instead of diarch structure of their stele have been reported (19). On a cross section of a root, xylem may not appear as a ring but in an irregular form (172), or even be broken into a number of parts (109). Roots have been reported (52), which, in addition to the usual cambial ring in the center, also had a number of additional small rings scattered irregularly throughout the region of the secondary phloem. It was suggested that it is this last "abnormal" structure which is the most desirable one from the agricultural point of view. In such a root addition of new cells and of new latex tubes can take place not as a result of the activity of only one, but of several cambial rings.

Latex tubes system. No latex tubes are found in any part of an ungerminated seed (21, 186). They begin to differentiate as soon as water is taken by a seed during germination. The first or primary latex tubes appear in the pericycle in close association with the phloem cells (19, 186), while the secondary latex tubes are differentiated in close proximity to the cambium (181). Latex tubes are produced by fusion of several cells in tandem at their cross walls (19, 21, 186) and by subsequent differentiation of the resultant fused cells. They are living structures containing cytoplasm and nuclei, the number and the shape of which vary according to age (24, 26).

Latex tubes of the same circle anastomose freely, though there is no connection between the tubes of different circles (19, 181, 186). It has been stated (152) that all the latex tubes of a kok-saghyz plant form a single continuous system, with the tubes of the leaves joining in the crown region on those of the roots.

It has been reported (151, 181) that practically all latex tubes of a mature root are formed and reach their maximum diameter before the peak of flowering is reached. In other words, at this stage the rubber-holding system of a kok-saghyz plant has attained its maturity. On the other hand, the greatest increase in diameter of a root as a whole occurs later, after the peak of flowering, and such

an increase is caused mainly by enlargement of the phloem parenchyma cells. As a consequence of this early development of its latex tube system and later growth of the root as a whole, there occurs after flowering a decrease in the number of latex tubes per unit area of the root. Since at the same time there also is not a decrease but an increase in the percentage of rubber in the whole root, it has been concluded that a considerable simultaneous increase in the concentration of rubber within the latex tubes must take place (151, 181).

Filippov (52) tried to correlate the diameter of a root with the percentage of rubber in it. He found that the percentage was inversely proportional to the diameter, and he explained this as being due to the lower number of latex tubes per unit of root area observed in roots of larger size.

Others (172) have described a definite gradient both in the number and in the average diameters of latex tubes in a kok-saghyz root. The smallest diameter and the smallest number of tubes per millimeter of root section are near the crown. Both these values increase toward the apex, reaching a peak 17.5 to 22.5 cm. from the crown, and then decline again. Such a gradient in diameter and number of latex tubes along the root is correlated with a similar gradient in percentage of rubber in both one- and two-year old roots, as reported in this and other papers (52, 169, 191).

PHYSIOLOGY AND BIOCHEMISTRY

Nitrogen and carbohydrate metabolism. Changes during the ontogeny of kok-saghyz plants in the first year of their growth were studied by Nichiporovich and Burovaya (150, 151). They found that an increase in dry weight continues until the very end of the growing season. In the period of growth before the peak of flowering has been reached, the plants produce mainly leaves. From the peak of flowering until the end of the growing season the bulk of the new dry matter is deposited in the roots.

In the period of their intensive growth, the leaves contain the bulk of the total nitrogen of the plant. From the peak of flowering until the end of the growing season the capacity of the leaves to synthesize proteins declines, and proteins already present in the leaves are partly hydrolyzed. Some of the products of such hydrolysis accumulate in the leaves, but a larger proportion is trans-

located to the roots.   As a consequence, the total nitrogen content
of roots by the end of the growing season is higher than that of
leaves.   Similar observations on the nitrogen metabolism in one-
(164, 185) and two-year (105) old plants were reported by other
workers.

Until flowering is reached the total sugar content of leaves is
relatively low, with the reducing sugars either equal to or slightly
in excess of the disaccharides.   After flowering the total sugar
content is increased and remains at a high level until the end of the
growing season.

Changes in the water-soluble polysaccharide of kok-saghyz roots,
which all Russian workers describe as inulin, have received con-
siderable attention.   The reducing sugar content of mature roots,
as compared with that of inulin, has been found to be low (79, 185).
The absolute amounts of inulin in one-year old roots were found to
increase continually throughout the whole growing season, with the
bulk of it being formed after flowering (79, 150, 151).   On the
other hand, the highest percentage of inulin both for one- (79, 150,
151) and for two-year old roots (105) has been reported at the
time of flowering, and it declines somewhat towards the end of the
growing season.   This is different from what is observed in the
ordinary dandelion, in which the highest percentage of inulin has
been reported (115) to occur late in the fall.

Using microchemical tests, Blokhintseva (21) has tried to localize
reducing sugars, inulin and rubber in one-year old kok-saghyz
plants which were in various stages of their ontogeny.   In leaves
this worker found reducing sugars only after flower bud formation.
In roots sugars and inulin were observed for the first time at the
time of flower bud formation.   At this stage both sugars and inulin
were present inside the root parenchyma cells.   Sugars were
particularly abundant around the latex tubes, while inulin was
spread throughout the whole phloem.   At flowering, large amounts
of sugars appeared inside the latex tubes, while inulin became
localized in the form of rings around the latex tubes.

Using similar methods of analysis, this worker (25) found that by
the middle of October kok-saghyz roots contained both inulin and
reducing sugars inside the parenchyma cells of both phloem and
zylem.   After two months of storage there was practically complete
disappearance of inulin and considerable accumulation of both
mono- and disaccharides.

Seasonal changes in rubber and resins. That the amount of rubber in a kok-saghyz root depends on the ontogenetic stage of the plant has been reported by a number of workers. According to Minbaev (135), in the first year of growth the percentage continuously increases until late in the fall, after which date there is a slight decrease. The rubber content of roots in the second year of growth, as a rule, is higher than in the first year. In the third year, the percentage goes down, which this worker attributes to age.

Mashtakov (123) followed qualitative and quantitative changes both in the rubber and in the resins of one- and two-year old roots in various stages of development. This worker reported continuous accumulation of rubber and decrease in resin content during the whole period of vegetative growth in the first year. The same was true for the rubber and resins produced in the the new tissues formed in the second year of growth. On the other hand, in the second year of growth the absolute amount of rubber in the "glove" remained fairly constant until the peak of seed formation had been reached, when it declined as a result of the sloughing-off process. The percentage of rubber in the "glove" had been, however, continuously increasing until the "glove" was sloughed off. This increase is attributed to the loss of dry matter in the "glove" itself (123, 125). The sum of changes in the new tissues produced in the second year of growth together with changes taking place in the "glove" determine the seasonal variations in the rubber content of the whole kok-saghyz root during the second year of its growth. From early spring until the peak of seed production there is a continuous increase of both absolute and relative amounts of rubber in a two-year old root. When the "glove" has been shed, the rubber content of such a root goes temporarily down, but it begins to increase again and continues to do so until the end of the growing season. According to this worker (121, 123, 125), the viscosity, molecular weight and degree of polymerization of rubber continuously increase during the normal life of both one- and two-year old roots. Depolymerization and quantitative decrease in the amount of rubber takes place only in the dying tissues, or in a "glove", and is due to the action of micro-organisms. With progress of the season both the absolute and relative amounts of resins decrease. At the same time their saponification, acid and ether numbers go up, iodine number remains fairly constant, and the index of refraction is decreased.

Blokhintseva (22) determined the refraction index of the rubber granules inside kok-saghyz latex tubes. She found that in younger plants and in those latex tubes which were closer to the cambium and consequently younger, the refraction index was higher. She suggested that a higher index is due to an admixture of resins, which in the earlier stages of development are present in larger amounts.

Nichiporovich and Bourovaya (150, 151) observed the greatest accumulation of rubber in a kok-saghyz root during the second or maturation stage of development. They suggested that by promoting such maturation one can stimulate accumulation of rubber in its root. These two workers (151) also suggested that resins in kok-saghyz might be by-products of general growth processes, probably connected with synthesis of proteins. They base their suggestion on the observation that the ratio between the amounts of resins and proteins in the roots remains practically unchanged throughout the whole vegetative period.

Synthesis of rubber. Nichiporovich (149) cites several examples to indicate that in a number of rubber-bearing plants the capacity to synthesize rubber is far in excess of the actual amounts of rubber present in them. He concludes, therefore, that the external conditions which bring about an increase in the rubber content of a plant do not promote the actual synthesis of rubber itself but act by stimulating an increase in the size of the receptacles in which rubber is stored.

As to the place of rubber synthesis in a plant, there exist two different opinions. According to one, rubber is produced in the mesophyll of a leaf and is transported as such to other parts. The latex tubes system is, therefore, simply a transportation system. According to the second opinion, rubber is produced not in the mesophyll of a leaf but within the latex tubes, probably from some carbohydrates. The latex system functions, therefore, both as a means of rubber translocation and as the site of its synthesis.

Zelinsky and Rapoport (213) suggested that isoprene is a "product of assimilation of carbonic acid under the action of light and chlorophyll as starch and carbohydrates in general". A close relation between illumination and synthesis of rubber has been reported by several workers (132, 153). From their observations on the diurnal changes in the rubber content of tau-saghyz leaves,

it has been concluded that rubber is produced in the mesophyll of leaves under the action of light, and that from the assimilating cells it is moved to the latex tubes and transported further (85).

According to Nichiporovich (149), since rubber is a highly polymerized substance, we can expect, on theoretical grounds, that its predecessors of lower degree of polymerization might have physical characteristics quite different from those of the final product. From the observation that many rubber-bearing plants contain in their assimilating cells substances which give various reactions characteristic of rubber, this author concluded that the bulk of rubber in plants is produced in the mesophyll tissue of their leaves. He suggests that such rubber is of low degree of polymerization, is easily soluble and is transported to other parts of the plant where it is polymerized further into insoluble rubber. This author concedes, however, that some true synthesis of rubber may also occur in some non-assimilating cells.

In both the palisade and spongy tissues of kok-saghyz leaves one can find round, glittering drops of inclusions which in Russian literature are called "mesecret" (mesophyll secretion). In order to determine whether these mesecret drops contain rubber, Blok-hintseva (23) investigated their composition in various stages of development. From the data obtained she concluded that mesecret drops in kok-saghyz leaves hardly contain rubber. In her later paper (24) this worker makes an even more emphatic statement that "synthesis of rubber in kok-saghyz is not connected directly on photosynthesis. Absence of rubber in the mesophyll secretion indicates, that synthesis of rubber does not take place in the assimilating tissues of the leaves".

According to Blokhintseva (24, 26), rubber in kok-saghyz is produced inside the living latex tubes. Since in comparison with that of a root the latex system of a leaf is developed to a considerably smaller extent, the bulk of rubber, according to her, is synthesized in the root. Having observed a protein covering on the outside of the granules of rubber, this worker thinks that rubber in kok-saghyz is formed in the cytoplasm as a result of the activity of plastids. The presence of protein covering on the granules of rubber is supported by Prokof'ev (173). Rubber granules in kok-saghyz are always spherical, and they increase in size with the progress of the season (224). Inside the old latex tubes, or under

pathological conditions (25), rubber granules coalesce and form rubber threads. In the first year of growth, such rubber threads appear for the first time after seed formation. Since no plasmolysis takes place in those latex tubes in which threads have appeared, Blokhintseva concluded that such latex tubes are dead and consequently have lost their capacity to synthesize more rubber. In support of her belief that rubber in kok-saghyz is formed not in the leaves in direct connection with photosynthesis but inside the latex tubes, this author also cites two other pieces of evidence: first—the appearance of rubber in a germinating seed, when cotyledons are still covered with the seed coat and before photosynthesis takes place (21); second—formation of rubber in seedlings which have germinated in darkness (24).

Prokof'ev (173, 231–233) also believes that rubber in kok-saghyz is produced in the latex system of its root and that this is done without any direct connection with photosynthesis. In support of his opinion he points out that rubber does not diffuse through the cell membrane and that once it has been formed in a cell it cannot leave it. Moreover, from the results of his own experiments with tau-saghyz, he doubts that latex tubes can function as a transportation system. He also quotes the experiments of Mazanko, in which tau-saghyz roots continued to synthesize rubber even after their leaves had been removed. Mashtakov (122) reported synthesis of rubber in kok-saghyz roots during their rest period, *i.e.,* when photosynthesis was not taking place. Neuman and Dobrovol'skaia (146) observed an increase in the rubber content of kok-saghyz roots during storage.

Physiological rôle of rubber. The question of synthesis of rubber in a plant is closely connected with that of its rôle. There is no uniformity of opinion on this subject, either. Some workers think that in a plant rubber serves some protective rôle; others look upon rubber as a reserve substance. Finally, a third group consider it to be a product of excretion.

According to Prokof'ev (173), rubber in kok-saghyz and a number of other plants cannot serve any protective rôle, as is indicated by the fact that numerous insects and cattle eat the plants. After an extensive survey of literature (174) Prokof'ev comes to the conclusion that rubber does not serve as a reserve substance either. Quoting from the experiments of Mazanko (126) on the

disappearance of rubber from the roots of tau-saghyz in starvation, Prokof'ev (174) concedes, however, that under some conditions rubber may disappear from plants. He makes, however, two qualifications: first, such disappearance occurs not under normal but extreme conditions of starvation; second, this disappearance may be only apparent, due to the change of an α-soluble rubber into an insoluble β-one (175). According to Prokof'ev (174), rubber is the end product of plant metabolism. That rubber is not an excretory product but a by-product of plant metabolism was concluded also by Pavlov (157).

According to Nichiporovich (149), the rôle of rubber in a plant may vary, depending on the degree of its polymerization, and rubber with low polymerization may serve as a reserve substance. In support of this opinion he quotes the results of experiments in which rubber disappeared on starvation of plants and reappeared either on illumination or on the injection of carbohydrates.

That rubber in a plant may be present in several forms whose rôles may also be different was suggested by Bosse (33, 34). According to this worker, the first step in the elucidation of the rôle of rubber in plants must be the working out of a better method of rubber analysis, which will permit one to differentiate between various forms of rubber in a plant.

Mineral nutrition. The effects of mineral nutrition on koksaghyz, in general, and on the accumulation of rubber, in particular, have received considerable attention from a number of workers. Since kok-saghyz is grown as an annual for rubber and as a biennial for both seeds and rubber, it has been suggested that experiments on its mineral nutrition cover not one but two years of its life (211). The majority of experiments reported so far cover, however, only the first year of growth.

Nichiporovich and Burovaya (151) found that the ash content of kok-saghyz plants, particularly of its leaves, is very high. This observation indicates high demands for inorganic constituents. While at the beginning of the season leaves contain eight to ten times more nitrogen than roots, toward the end of the season the total amounts of nitrogen present in the roots and leaves are about equal. Leaves also contain about three times as much calcium and potash as do roots. On the other hand, the amount of phosphorus in roots is very high, being slightly higher than in leaves, even at

the beginning of the growing season. By the end of the season it becomes practically twice as high in roots as in leaves.

It has been reported by several workers (4, 76, 77, 78, 185) that in the earliest stages of kok-saghyz ontogeny, which corresponds to the stage in which a rosette of leaves is formed, there must be present fairly high amounts of N in the soil. That large amounts of N in this stage of plant development have a depressing effect has also been reported (39, 78, 80, 116). Increased amounts of N must be available to kok-saghyz during flowering and seed formation (4, 77). In the stages from completion of seed production until the end of the growing season, the amounts of N should be lowered again (82). Large amounts of it in this last stage of growth will stimulate production of leaves at the expense of roots (76, 77, 78). While some reports (116, 185) indicate that accumulation of rubber in kok-saghyz is more active on NO_3 than on NH_4, according to the more recent work this may not always be true. Thus it was reported (77, 82, 112) that roots had a higher percentage of rubber in them when the plants received N as NH_4 rather than as NO_3.

From the observation that phosphorus stimulates biological maturation of kok-saghyz, Nichiporovich and Burovaya (151) have recommended that increased amounts of it be given after the peak of seed production. This conclusion has been supported by other workers (4, 78). On the other hand, it has been pointed out (39, 78, 116, 130, 185) that a high level of phosphorus nutrition should also be present in the earliest stages of development.

It has been reported by several authors (116, 117, 145,) that addition of K decreases the rubber content of roots. On the other hand, Kalinkevich (77, 78) points out that this is true only when in the later stages of ontogeny the level of the N nutrition is too high. When this level is low, addition of K increases the longevity of the leaves and brings about an increase and not a decrease in the amount of rubber in the roots (77, 78, 185).

That kok-saghyz responds favorably to several trace elements (84), radio-active elements (42) and calcium (84, 117, 129) has been reported. The amounts of various trace elements in kok-saghyz have been determined (31).

Several workers have tried to determine the effects of mineral nutrition on plants, in general, and on kok-saghyz, in particular,

one is constantly finding emphasis laid on the two following points: first, that the needs of a plant for various elements is not constant throughout its whole ontogeny, but varies, depending on the stage in which the plant is (38, 39, 76, 77, 78, 80); second, that the after-effects of a shortage or an excess of an element early in ontogeny may have pronounced effects also in later stages of growth (77, 78, 80, 164).

Several workers have tried to determine the effects of mineral nutrition on the physiological and biochemical characteristics of kok-saghyz plants. Thus it has been reported (79) that an increase in the end content of the soil brings about an increase in the $\frac{\text{reducing sugars}}{\text{invert sugars}}$ ratio of leaves in any stage, while a higher K content increases the relative proportion of cane sugar (76).

In drawing conclusions from the results of analysis on the effects of various elements on the biochemical characteristics of a plant, one should remember, however, that the same element may have different effects, depending on the ontogenetic stage of the plant. Thus there is a statement that "increased amounts of N fertilizers bring about even a decrease in the amounts of carbohydrates" (145). On the other hand, Kalinkevich (80) has published data to indicate that in the early stages of ontogeny higher amounts of N fertilizers brought about an increase and in the later stages a decrease in the total sugar content of kok-saghyz leaves.

Water content of the soil. That kok-saghyz in its native habitat prefers a high percentage of water in the soil has been pointed out (109). It has been reported (103) that the higher the water content of the soil is, the greater the final dry weight of the plants, the higher their percentage of rubber and the shorter the time before appearance of flowers. The greatest need for water is in the first period of growth up to the time of flowering. Observations made by these two workers have been confirmed (214).

Summer dormancy. One of the biological characteristics of kok-saghyz is its temporary period of rest in the middle of summer in the second year of its growth. It was suggested (205) that a strong tendency towards a resting period occurs after flowering and formation of fruits. Since at this stage the synthetic activity of its leaves decline and there occurs an accumulation of various soluble substances which are at least partially translocated to the roots, one

expects theoretically to find at this stage of growth an increase in the osmotic pressure of the cell sap of both leaves and roots. Such an increase has been observed (205). It was also reported (205) that dusting plants with chalk or watering them slows down the rate of increase in their osmotic pressure and delays the onset of their period of rest. Removing flower buds from some two-year old plants and leaving them on others, Lebedeva (105) found that such treatment only delayed the onset of the period of rest but did not eliminate it completely.

On the other hand, it was suggested (147) that the summer rest of kok-saghyz is caused not so much by the fact that the plants have then reached a definite stage of their ontogeny but by unfavorable external conditions. In support of such an opinion these two workers (147) cite their experiments on both one- and two-year old plants which grew under favourable external conditions and did not enter rest periods, while comparable plants growing in the fields did. Since leaves of kok-saghyz are sensitive to high temperature and insolation, a decrease in these factors theoretically should also eliminate a rest period. They also cite experiments in which dusting or shading with cheese cloth either cut down the number of plants entering a rest period or completely eliminated it.

ANALYTICAL METHODS FOR DETERMINATION OF RUBBER

The method of analysis which has been very widely used by Russians in their work with rubber-bearing plants is that of bromination. This microscopic method was orginally a qualitative one (170), though later it was made quantitative (171). It has been used particularly in field work and where the facilities of the usual chemical laboratory were not available. Kostriukova (96) has modified the method for studying the rubber content of leaves. Blokhintseva (22) has combined this bromination method with that of Becke for the determination of the refractive index of solid substances. Ignat'ev (35) suggested cold extraction of air-dried plant tissues with chloroform. Such an extract can be prepared in the field and sent to a properly equipped laboratory to be analyzed for its rubber content. In order to follow the rate of rubber extraction from the roots in a factory, Izriumov (74) suggested a method for quick determination of rubber, using both a solvent for extraction of the rubber and a centrifuge to remove the non-soluble residue.

In selecting kok-saghyz roots with the highest rubber content it is necessary to analyze large numbers of plants in a short period of time. Several different methods have been suggested for this purpose. Stolbin (201) proposed a quick method based on the digestion of roots first in 3% NaOH, then in concentrated H_2SO_4, and finally determining gravimetrically the film of rubber so isolated. Koialovich (91) improved Stolbin's method by taking samples for analysis from a definite region of the root, by previous drying of the samples to eliminate loss of latex, and by eliminating digestion with concentrated H_2SO_4, which affects the rubber itself. Goriainov (66) suggested an electrometric method based on the determination of the E. M. F. in the living roots. A quick method, based on the appearance of rubber threads between the two ends of a broken air-dried root, has also been suggested (1, 197). This last method is recommended in mass selection of kok-saghyz roots according to their size.

CULTIVATION

From an examination of the various rubber-bearing plants which can be grown in the Soviet Union, various workers (7, 8, 43, 54, 183, 184) have concluded that kok-saghyz is at present the most promising plant. It greatly responds towards the improved conditions of cultivation and can stand a variety of climatic conditions. Potentialities of kok-saghyz cultivation seem to compare favorably with those of *Hevea* (192).

Numerous books and booklets (1, 10, 15, 55, 92, 98, 118, 128, 148, 168, 197), papers (6, 8, 138, 154, 183, 184, 200, 215) and pamphlets (40, 41, 113, 114, 199) supply instructions on the cultivation of kok-saghyz, and several authors (12, 20, 106, 131, 161) have described their experiences in growing the plants. Since cultivation in many respects is similar to that of sugar-beet, many practices used in the cultivation of the latter have been adopted for kok-saghyz without any modification (203).

Practically all authors emphasize the importance of choosing proper soil and proper predecessors (200). Kok-saghyz requires rich, not too heavy soil, and does best on peat and muck. For this reason White Russia, with a large percentage of its land under peat-bogs, has been considered an important potential region of U.S.S.R. for future cultivation of kok-saghyz (2, 14, 83, 128). Since the plant does not like an acid medium (117), such soils must be heavily limed (129).

Growing very slowly in the first three or four weeks of its life, kok-saghyz requires soil free from weeds. For the same reason it is recommended to weed its plantations and to cultivate between the rows at least four or five times during the growing season (13), and to dig out other species of dandelions at least twice. Minbaev (136) compares various characteristics of kok-saghyz with those of the common dandelion. According to his data, the common dandelion has a far greater vitality than kok-saghyz and on cultivated fields is a far stronger competitor of it than any other weed.

Having roots which penetrate into the soil to a great depth, kok-saghyz requires deep plowing. For spring sowing, plowing should be done in the fall, while for fall sowing it should be done four to six weeks before the sowing. It was reported that in the spring kok-saghyz seeds begin to germinate when the temperature reaches 5° to 10° C. (142), though for uniform and rapid germination they require temperatures between 25° and 30° C. (210). Since the usual temperature at the time of spring sowing is much lower than 25° C., dry seeds sown in the spring do not germinate uniformly. If, however, they are sown in the fall, remain in the ground during the whole winter and germinate only the following spring, then, provided other conditions are satisfactory, they germinate quite uniformly. For this reason sowing in the fall was recommended in the earlier instructions (16, 98, 107, 113). On the other hand, fall sowing has several drawbacks. Too early sowing in the fall results in the seeds germinating the same fall. As a consequence, a large proportion of young seedlings are killed during the winter, although mature roots can stand temperatures as low as −29° C. (48). Too late sowing in the fall precludes proper covering of seeds with soil, and this again cuts down the number of seeds germinating the next spring. Moreover, stratification of seeds suggested later resulted in their uniform germination even at the low spring temperatures. For these reasons spring sowing is recommended in later instructions (1, 10, 194, 197).

It was pointed out by Lissenko (110, 111) that cultivation of the soil immediately before sowing has been the main reason why spring sowings with stratified seeds have yielded poor results. He noted that such cultivation dries the upper layer of the soil in which small kok-saghyz seeds are deposited during sowing, and for

this reason the seeds can not germinate properly. The more recent views on how to carry out spring sowing of kok-saghyz have been given by Zasiadnikov (212). Since kok-saghyz seedlings are very weak, they break through the soil only with great difficulty. Sprinkling freshly sown rows with humus has been recommended to prevent formation of the soil crust and to insure more uniform appearance of the seedlings (163). It was suggested earlier (49) that if kok-saghyz seeds are incorporated into small clay balls and such balls are sown in the field, then the seedlings grow in small clusters and penetrate the layer of the soil above them with greater ease. Zasiadnikov (212) describes an apparatus to prepare such clay balls.

Though it has also been recommended (10, 51, 110, 212) to start new plantations by means of root cuttings, the results of experiments on the vegetative propagation of kok-saghyz obtained by different workers do not agree. Some (109, 133, 220) were successful, others (141) had considerable difficulty. A promoting effect of various growth substances on the percentage of cuttings rooted has been reported (119).

Extensive use of fertilizers, even on peat soils (56), has been recommended by all authors, and it has been suggested that fertilizers be given on at least three different occasions (198): first, organic and mineral fertilizers introduced during the deep plowing of the soil; second, mineral fertilizers introduced either before or simultaneously with sowing of the seeds; third, several introductions of various mineral fertilizers during the growing season itself. The last type of fertilizers is often introduced dissolved in water. The beneficial effects of introducing inorganic fertilizers during the growing season have been described by several workers (81, 140).

First-year plantations are dug late in the fall before the period of rains. During the first year of their growth, Altukhov (9) observed a continuous increase in the total fresh weight of roots and in the percentage of their rubber until the end of the growing season. From such observations he recommended digging such roots late in the season. According to him, the amount of rubber added during the second year of growth does not warrant leaving a well developed one-year plantation for the second year.

In the earlier years of kok-saghyz cultivation, when there was

a great shortage of seeds, they were collected even from one-year
old plantations. In later years, when there was a fair supply,
there has been a tendency not to collect seeds from one-year old
plantations at all but to use the latter only as a source of rubber.
Seeds have been collected recently only from plantations left for
the second year. This change is due to the fact that seed collec-
tion is the most labor-consuming single operation in the enter-
prise, the cost of which may amount to 50% of the total cost (162).
In the meantime, while the average seed production from one
hectare of a one-year old plantation is three to five kilograms, a
two-year old plantation yields 30 to 40 kg. per hectare (136). The
greatest yield of seeds from a two-year old plantation has been
reported as 215 kg. per hectare (136).

The yield of fresh roots per hectare of a one-year old plantation
varied from zero to the highest value of 112 centners (one cent-
ner = 100 kg.) on a drained bog in White Russia (5). It is
interesting to note that the Soviet Government (67) gives bonuses
to the growers of kok-saghyz when their yields of fresh roots per
hectare of one-year old plantations exceed 10 to 13.5 centners,
depending on the region of cultivation. The date for digging
roots from a one-year old plantation varies from the end of
September until the middle of October, depending on the year and
the locality.

On a kok-saghyz plantation in the second year of its life, flower-
ing begins about June 1 and lasts until the middle of July, the
exact dates varying from one geographical location to another.
Each plant usually produces 30 to 40 flower heads within 10 to 15
days. A flower head is picked when it becomes cylindrical; it
acquires a yellowish color then and the white pappus hairs become
visible (1, 10, 197). Collected flower heads are dried and the
pappus hairs removed from the seeds.

It is recommended to dig two-year old plantations after the peak
of flowering and before the roots have shed their "gloves" (1, 11,
73, 197). In order to determine the best time for this in various
regions of U.S.S.R., it was decided to carry out extensive tests in
the summer of 1943. Operators of various commercial plantations
were requested to take samples early in the spring, at the peak of
seed formation, after the end of seed formation when the plants
began to enter their period of rest, and again after the plants had

entered their rest period. A special pamphlet (18) gave instructions as to how to take samples and either to analyze them for their rubber content locally or to send them to a properly qualified laboratory.

Roots are lifted from the soil usually by a modified sugar-beet lifter or an ordinary plow, and are removed by hand. Their leaves are cut off and the roots sent to the factory either fresh or air-dried. Roots dug in the summer are usually sent in air-dried. Those dug in the fall are usually sent fresh. While waiting to be processed they may be stored in pits, though their conversion into a silage (204) has been suggested.

One of the greatest handicaps in the cultivation of kok-saghyz is its demand for a great deal of labor, and so mechanization of its cultivation is one of the first tasks to be solved (65, 190). While equipment suggested for tau-saghyz (155, 156, 189) can also be used for kok-saghyz, such machines are not very satisfactory. Suggestions on the type of a seed drill to be used for kok-saghyz have been made by several authors (1, 47, 179, 197). Filippov (53) described briefly a machine to collect seeds, and a detailed description of this machine is given elsewhere (1). An ordinary plow and a sugar-beet lifter have been used to dig the roots, but a special machine for the purpose has been suggested (17).

<div align="center">PATHOLOGY</div>

A general description of the various parasites found on roots, leaves and seeds of kok-saghyz are given in various instruction booklets on how to grow the plants (1, 10, 197). There is one list of parasitic insects (59) and another describing those found on seeds and flower heads (63). Ghilarov (58) came to the conclusion that *Olibrus bicolor* F. and *Ceuthorrhynchus punctiger* Gyll are the two most important seed parasites. Since these two insects come to kok-saghyz from the common dandelion, he suggested extermination of the latter in the vicinity of the former, as one of the most important methods of control. In a more recent paper (62) this author also suggested dusting flower heads with naphthalene. An infestation by a parasite coming from *Brassica Napus* has also been reported (217).

According to Ghilarov (57), considerable destruction of various root rubber-bearing plants may be caused by root aphids and ants.

Skabrilovich (195, 196) described nematodes on kok-saghyz roots and reported (195) that in one case he observed infection up to 33% of all the roots examined. Root-rot on 15% of the plants examined was also reported (187). Emelianova (44) recommended chloropicrin (nitrochloroform) as a sterilizing agent to control various soil parasites. Two different species of *Orobanche* have been reported on kok-saghyz (61).

That kok-saghyz seeds may be infected with a number of fungi which later may seriously injure or even kill the developing seedlings, has been noted (86), and to control such fungi, treatments with arsenic (87) and copper (202) have been suggested. Rischkov (177) described a virus disease.

SELECTION

Having observed considerable polymorphism of kok-saghyz plants growing in different localities, Lipschitz (109) suggested that it may be due either to environment or to the existence of several genetically different forms. To test this point, Bulgakov (36) grew plants under identical conditions, using seeds from mesophytic, hygrophytic and xerophytic forms collected from different natural habitats. Since no significant differences were observed among the plants studied, this worker concluded that polymorphism is caused by environment. When seeds collected from the wild were sown side by side with those from cultivated plantations, their germination was less uniform and the roots produced were considerably smaller (64).

Kupzow (99) recommended using on kok-saghyz mainly the mass selection method, since in the early stages of selections such a method gives quicker results than the slower one of individual selection. Mass selection of large roots and their subsequent use to start new plantations for production of seeds is the standard procedure recommended by numerous authors (1, 10, 197). Since Kupzow observed plants with large roots to have pinnately lobed and divided leaves, he suggested selecting such plants and not those with simple leaves (99, 100, 102). According to this worker (102), the presence of specimens with different forms of leaves in the same plantation of one-year old plants indicates the existence of at least two genotypically different races. Some plants go through their ontogeny quickly and soon enter their later stages characterized by

pinnately lobed leaves. Such plants also flower usually the first year (101). Others go through their ontogeny slowly, they develop pinnately lobed leaves only under very favourable conditions and flower usually the second year. Work by several authors (50, 104, 134) has cast doubt on the value of using morphological characteristics of leaves as a yardstick in the selection of kok-saghyz.

It was also reported that only plants with medium size roots flower the first year, while those with the largest roots flower only the second year. On the strength of this observation several authors (88, 95, 99) have suggested that selection be made for plants which do not flower the first year.

Kok-saghyz can be hybridized only with other diploid and not with polyploid species of *Taraxacum* (159). Since such hybrids, even when established, are nearly completely sterile and are characterized by a lower rubber content, "distant hybridization is hardly advisable in breeding *T. kok-saghyz*" (159). Similar conclusions were also reached by others (93). It was stated (160) that the average setting of fruits in hybrids between kok-saghyz and other diploid dandelions amounted to only 2% to 3%.

Tetraploid kok-saghyz plants were produced by immersing into a solution of colchicine either seeds (218) or young seedlings (143). Reproducing tetraploid plants so formed by means of root cuttings, it was possible to propagate them further by seeds (144). Tetraploid kok-saghyz plants are reported to be of larger size, in general, with increased diameter of latex tubes in their roots and twice the weight of seeds as compared with those from diploid plants (144).

Koroleva (95) recommended that kok-saghyz plants be selected on the basis of root weight, percentage of rubber, and the weight of seeds. Among indirect desirable characteristics, this worker lists absence of flowering the first year and divided leaves. According to her, selection and favorable agricultural practices are the two most powerful factors in changing kok-saghyz from a wild into a cultivated plant. Discussing ways and means of changing it into a cultivated plant, Minbaev (136) also stresses the importance of favorable conditions during growth and recommends reducing the number of plants per hectare to not more than 300,000 or 400,000.

In discussing selection of kok-saghyz, Filippov (52) brings forth data to indicate that the most desirable form is not a single but a branched root. According to him, such a form contains a higher

percentage of rubber. He also describes roots with modified anatomical structure containing several additional cambial rings, and believes, along with others (165, 227), that the future cultivated forms of kok-saghyz should have such a modified anatomical structure with additional cambial rings.

According to Rudenskaia (182), from the anatomical point of view a large single root with latex tubes of large diameter is the most desirable. Her opinion is based on the observation that the larger a root is, the greater is the number of its latex tubes. On the other hand, the percentage of rubber in a root is correlated with the mean area of its latex tubes.

Minbaev (139) has suggested a new method of kok-saghyz breeding from the following considerations given in his earlier paper (137). The flowering period in both one- and two-year old plants is extended for several weeks, and this means that early flowers are produced under different external and internal conditions than the late ones. This is equally true for the florets in various circles of the same head, since they do not open all at once but within one or even several days. On the strength of this observation he suggested that the kind of gametes produced by a plant will vary, depending on the time of day or season when they are produced. He gives data to indicate that seeds collected in September produced plants with higher rubber content than those collected in August; also, that seeds collected from different circles of the same head had different characteristics which varied in a regular manner.

In spite of all work done on kok-saghyz so far, this plant is still represented essentially by its wild forms. There are only two references in the literature to improved forms. In one (216) a brief mention is made of two varieties with 15.5% and 16.5% of rubber, respectively. In the other (219) are described two varieties differing one from another in a number of characteristics.

TECHNOLOGY

Since rubber in a kok-saghyz root may be present either as latex or as coagulated threads (25), attempts have been made to extract both of these forms separately. In his first paper on this subject, Ignat'ev (68) described a method of extraction by which he was able to obtain up to 40% of the total rubber in the roots.

Low concentration of rubber in the latex and its instability on standing were the two main difficulties experienced by this author. In his later paper (69) he described several improvements of his original method and suggested a number of problems which should be studied. Siniavskiĭ (191), giving the principle of latex extraction from kok-saghyz, suggested that even up to 50% of the rubber in the roots might be removed as latex. Cutting of kok-saghyz roots into small pieces is one of the first stages in the extraction, and Vasil'ev (208) investigated this stage from the mechanical point of view.

Ignat'ev, Uzina, and Erofeev (72) studied the effects of conditions during storage of kok-saghyz roots upon subsequent extraction of latex from them. They report that one-year old roots could be stored and used as a source of latex for six months during the winter, though the temperature of $-12°$ C. brought about coagulation of the latex in the roots within a few days.

The total rubber in the roots of kok-saghyz or the residual amount left after extraction of latex, can be obtained in several ways. Bobkov (29) suggested that cut roots be digested with sodium hydroxide in order to break the cells and to liberate the coagulated threads of rubber. Since the specific gravity of the particles of rubber is somewhat lower than that of other suspended parts, they can be separated from such a mixture by means of a centrifuge. Using this method, about 75% of the total rubber in roots is extracted and 25% lost. In order to cut down these losses, Bobkov (30) suggested the use of a number of special traps and reported that with the help of these the total extraction can be raised from 75% to 85% or 90%.

Tverskaia and Iossa (207) pointed out a number of disadvantages of the method suggested by Bobkov. They say it is complicated, expensive, dangerous on account of the use of a centrifuge, leaves a considerable percentage of rubber unextracted and, finally, affects the quality of the rubber extracted because of the alkali involved. For all these reasons they suggested the use of a mechanical method.

Kogan (90) objected to the construction of factories for extracting rubber by the mechanical method as suggested by Tverskaia and Iossa. He based his objections partly on the ground that this method is less familiar in Russia than the one described by Bobkov.

His main objections are, however, that the mechanical method precludes the utilization of inulin present in the roots for the production of alcohol, and he also claims that a centrifuge properly designed is a more efficient equipment than a ball mill.

Break-down of the root tissues for liberation of the rubber by means of micro-organisms has also been suggested by several authors (29, 90, 207). Though this method is quite simple, it requires large containers for the digestion, precludes utilization of inulin, and imparts a strong unpleasant odor to the final product. While extraction of rubber by means of solvents has been investigated for at least one of the saghyzes (176), no data have been found in the literature to recommend its use for kok-saghyz.

Fabritsiev and Vishnevskaia (45) extracted rubber from one-year old roots and studied its chemical and physical charasteristics. They reported that the initial relative viscosity of the non-plasticized pale crepe is two and a half times as high as that of rubber from kok-saghyz. After plastication for 21 minutes the relative viscosity for the pale crepe dropped to one-fifth of its original value, while that for kok-saghyz was slightly less than a third of its original value. The data obtained for the capillary rise of the rubber extracts in benzol were in agreement with those for the viscosity.

The technological characteristics of rubber from kok-saghyz were described and compared with those of imported natural rubber by Fabritsiev and Vishnevskaia (46). They studied the behaviour of kok-saghyz rubber alone and in mixtures with lamp black, coaolin, chalk and various softeners.

Ignat'ev and Ustimova (71) removed resins from crude kok-saghyz rubber, prepared a solution of pure rubber hydrocarbon isolated also from kok-saghyz, and studied the effects of adding various amounts of resins to a solution of such pure rubber. The viscosity of the rubber solution has been taken as an indicator of such effects. They reported that addition of resins lowers the viscosity of the gel but not of the sol solutions. For this reason they suggested that the molecular weight of rubber in a sol solution could be calculated according to the formula of Staudinger, whether resins are or are not present.

From their studies on the swelling of kok-saghyz rubber in various solvents, Ignat'ev and Senatorskaia (70) have concluded that the higher the degree of polymerization of rubber, the greater is its

swelling in a solvent. From the observation that a plasticized rubber has a lower capacity to swell, they suggested that plastication of rubber decreases the degree of its polymerization. In evaluating all the data on the physical characteristics of rubber from kok-saghyz, one should keep in mind the observation (121, 123) that such characteristics are not constant but vary, depending on the ontogenetic stage of a plant from which such rubber has been extracted.

Only fragmentary information is available on the actual utilization of rubber from kok-saghyz for manufacturing various articles. Thus Rogov and Magidov (178) reported that tires and inner tubes made from it differed but little, according to their mechanical characteristics, from those made from imported natural rubber. According to Shelepina (188), rubber from kok-saghyz could be substituted for smoked sheet in the production of glue, and the same is true in the production of rubber threads (206).

While rubber is the main product for which kok-saghyz was originally introduced into cultivation, its reported inulin content of up to 40% of the dry weight of roots makes this plant also desirable for the alcohol industry (27, 97). Thus a number of authors have suggested (28, 72, 90) that previous to the extraction of rubber inulin and sugars be removed from the roots and fermented into alcohol. It was reported that a ton of fresh roots should yield 60 to 65 liters of alcohol (29, 72), or for one ton of rubber extracted one should get about two tons of alcohol (90). Bobkov (28) suggested that in order to hydrolyze inulin present in kok-saghyz roots an acidity of at least pH–2.5 should be arranged. In his experiments on the fermentation of the roots, about 90% of the soluble carbohydrates present initially in the roots were fermented.

SUMMARY

Kok-saghyz is·reported to be one of the most promising rubber plants which have been found within the last few years. It was discovered in 1931 in the central Asiatic regions of U.S.S.R., close to the Chinese border. Being in cultivation only for the last 12 years it is still fairly close to its original wild type, and its improved forms are just beginning to appear. This fact, together with its great demands for labor and the absence of specially designed machinery, represent the greatest difficulties in its present

day cultivation. Kok-saghyz, however, is a plant which seems to have considerable biological potentialities, and it responds greatly to the improved conditions of cultivation.

Kok-saghyz contains rubber in its roots, and the recent tendency in its cultivation is to grow it either as an annual or a biennial, depending whether one wishes mainly its roots or seeds. As a source of rubber, kok-saghyz is sown usually in the spring, and its roots are dug up late in the fall of the same year. As a source primarily of seeds, a plantation is left for the second year. Seeds are collected by the middle of the second season of growth, and roots are dug up shortly after the peak of seed production has been reached. Extraction of rubber from kok-saghyz roots does not present any serious difficulties.

Since kok-saghyz roots contain up to 27.89% of their weight as rubber, the main interest in this plant so far has been as a source of rubber. It should be remembered, however, that up to 40% of its dry roots is in the form of an easily hydrolyzable polysaccharide which can be fermented, and for this reason kok-saghyz may serve also as a raw material for the alcohol industry.

SUPPLEMENT

The following changes in the foregoing account have been necessitated by literature which could not be included in the original writing.

Page 420. Delete last line and its continuation, and substitute:

Sexual reproduction is normal (158), with cross-pollination as the usual method. In native habitat of kok-saghyz, pollination has been reported (226) as carried out mainly by the burrowing bee (*Halictus*), while under the conditions of cultivation it is effected by

the ordinary honey bee (*Apis*). Occasional cases of self-pollination have also been reported.

Page 424, second paragraph. Add:

Under some conditions the individual vascular bundles of such a modified root may separate, and by the end of the growing season the whole root becomes transformed into a cluster of smaller root-lets (225).

Page 424, third paragraph. After "cambium (181)" insert:

Differentiation of the latex tubes in each circle is stimulated by the presence of young leaves, which are growing under favourable light conditions (230).

Page 424, fourth paragraph. After "circles (19, 181, 186)" insert:

According to Nichiporovich and Ivanitzkaja (229) the number of such circles in a root is closely connected with the number of leaves.

Page 430, second paragraph. Delete last two sentences and substitute:

In his earlier paper (122) Mashtakov reported synthesis of rubber in kok-saghyz roots during their rest period. The same author showed later (228) that such an increase is only relative, being due to the increase in the dry weight of roots. The absolute amounts of rubber remain the same. Neuman and Dobrovol'skaja (146) observed an increase in the rubber content of kok-saghyz roots during storage. Such an increase was promoted by the conditions of impeded gas exchange (223, 234). It was also stimulated by wilting, and checked when roots were kept at constant humidity (223).

Page 441. Delete fourth paragraph and substitute:

Tetraploid kok-saghyz plants were produced by immersing into a solution of colchicine either seeds (218, 235) or young seedlings (143, 222). Such plants are usually of larger size, with the increased diameter of their latex tubes (144) and higher amounts of rubber per root (235).

<div align="center">LITERATURE CITED[1]</div>

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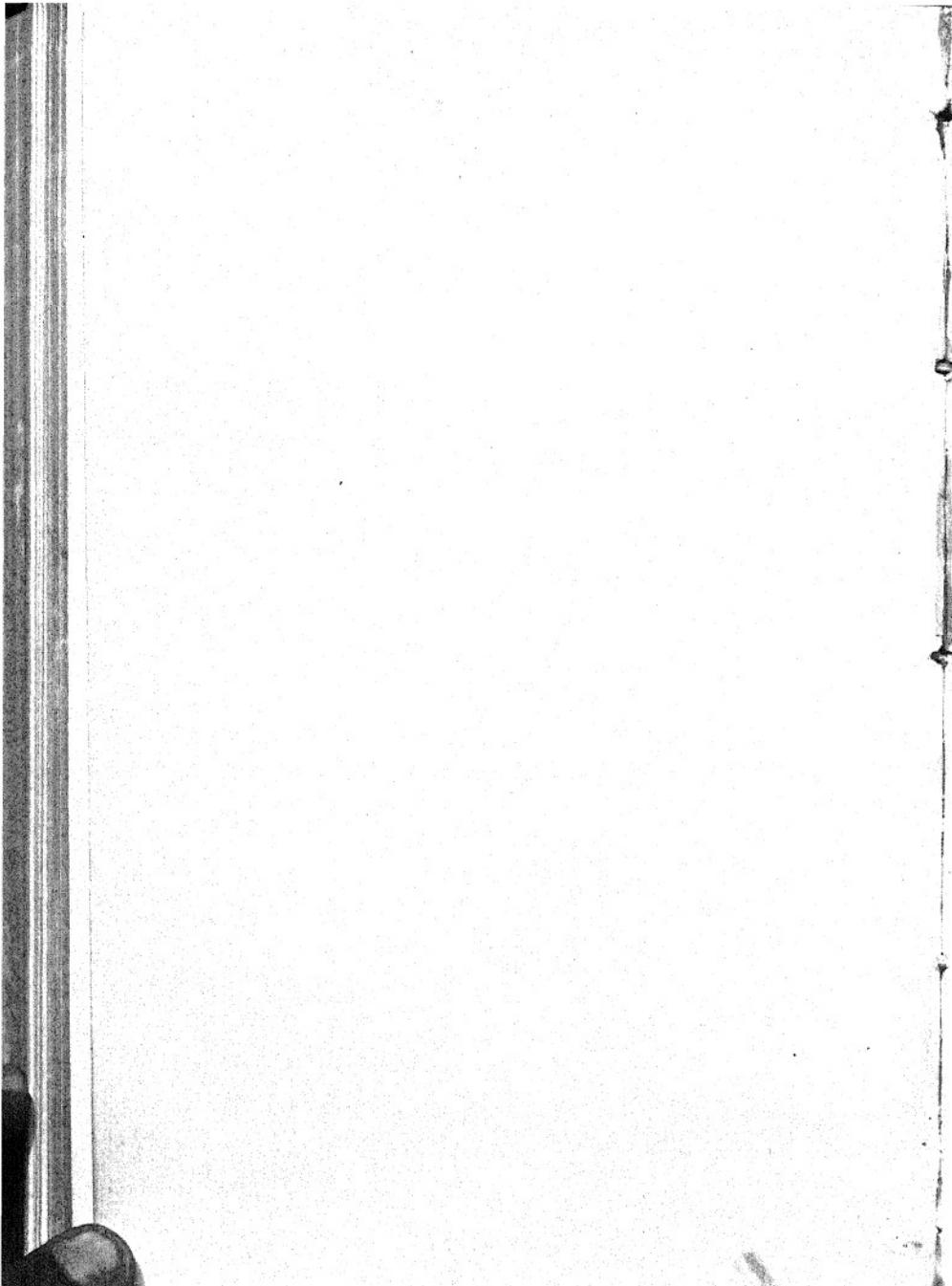
THE BOTANICAL REVIEW

THE CYTOLOGICAL ANALYSIS OF SPECIES HYBRIDS. II[1]

G. LEDYARD STEBBINS, JR.
University of California

The present article is intended as a supplement to the admirable one on the same subject written by Dr. Karl Sax for the first volume of this journal (126). During the last ten years many papers on hybrid cytology have been written, and our understanding of species relationships in terms of the chromosome behavior of their hybrids has greatly increased. In particular, certain genera, such as Gossypium, Triticum-Aegilops, Layia, Datura and Nicotiana, have been so intensively studied that hybrids are known involving a majority or practically all of their species. With such knowledge available, cytogeneticists can confidently establish principles concerning the reasons for the chromosomal behavior found in various types of hybrids, and even for the nature of the genetic barriers between species. That age-old problem of biology, what makes species distinct from each other, is gradually being solved by the application of the tool forged for us by the cytologists and geneticists of ten to twenty years ago, and termed by Sax the "permanent hybrid-cytogenetics".

FACTORS INVOLVED IN CHROMOSOME PAIRING

This new knowledge, however, has made necessary very little revision of the general statements made by Sax. His section on "factors involved in chromosome pairing" is as valid today as it was ten years ago. Failure of pairing due to genetic factors, or "asynaptic genes", has been found, in addition to Zea and other examples cited by Sax, in Rumex acetosa (166), Pisum sativum (49), Nicotiana sylvestris (45), Oenothera (17), Gossypium hirsutum × barbadense (10), Allium amplectens (75) and Triticum aestivum (78). Li, Pao and Li (78) have suggested that the

correct term for this phenomenon is "desynapsis", since in every case where the necessary observations have been made the chromosomes actually are synapsed during early pachytene, and fall apart during late pachytene and diplotene. Genetically controlled abnormalities of spindle behavior have been found in *Zea* (18), *Saccharum* (59), *Lathyrus* (153), in the hybrid *Mentha piperita* × *spicata* (147), and in a *Festuca-Lolium* hybrid derivative (30). In *Lathyrus* the abnormal stretching of the spindle is accompanied by an abnormally great contraction of the chromosomes, a reduction in the number and an increase in terminalization of the chiasmata. In *Secale cereale*, Müntzing and Prakken (95) found occasional sporocytes at meiosis with a larger or smaller number of bivalents than the seven which were to be expected on the basis of the known somatic chromosome number of the plant. This was explained on the basis of an exchange of chromosomal material between archesporial cells in the premeiotic mitoses, or cytomixis. These examples show that genetically controlled abnormalities of meiosis, apart from those produced by lack of structural homology in the chromosomes, are likely to be found occasionally both in good species and in hybrids. Nevertheless, they are rarely if ever the principal cause of cytological irregularities and sterility in species hybrids. In most hybrids between closely related species, the behavior of the spindle and related structures is normal, and the chromosomes are partially or nearly completely paired at prophase and metaphase of meiosis, with subsequent lagging, bridge-fragment configurations and other phenomena now definitely associated with lack of homology. Non-homologous pairing at meiosis has not yet been recognized in any plants besides *Zea*, as reported by Sax (126), so that the presence of pairing and chiasma formation in species hybrids can still be taken as a very probable indication of homology between the parental chromosomes.

FERTILE SPECIES HYBRIDS

The problem of fertile species hybrids must now be considered on the basis of recent definitions of the species, such as those of Clausen, Keck, and Hiesey (20), and of Dobzhansky (34). No less than eleven such definitions, by different authors, are included in the anthology on "The New Systematics", edited by Huxley (57). These authors all agree that isolation barriers of some sort

are essential to build up and maintain the morphological distinctness that enables us to recognize species, and that the sterility of pollen or seed in otherwise vigorous F_1 hybrids is one of the most important of these barriers. All, moreover, are further agreed that two species may under some conditions be effectively isolated from each other even though the F_1 hybrid between them forms perfectly normal pollen and sets a high percentage of seed. Dobzhansky (34, *257*) and Huxley (57, *11*) recognize the importance of physiological isolating mechanisms, such as ecological, seasonal or temporal isolation, wherein the parental forms do not meet. Clausen, Keck, and Hiesey, rejecting these as valid criteria for keeping species distinct, recognize only genetically controlled barriers to gene exchange. They point out, nevertheless, that the sterility of F_1 hybrids is only one of these barriers, and that the weakness or sterility of F_2 segregates, due to their possession of disharmonious combinations of genes from the two parents, must also be considered. They cite as examples of species hybrids which are fertile in F_1, but in which the barrier to gene exchange becomes evident in later generations, *Zauschneria cana* × *septentrionalis* (21), *Layia gaillardioides* × *hieracioides* (22) and *Solidago rugosa* × *sempervirens* (47). Other such examples are *Gossypium arboreum* × *herbaceum* (56, 130, 159) and *Solanum Henryi* × *verrucosum* (114). In addition to these and the genera cited by Sax (126), fertile, cytologically normal F_1 hybrids between species generally recognized by systematists are known in *Antirrhinum* (38), *Aster* (161), *Canna* (101), *Catalpa* (136), *Ceanothus* (81, 82), *Crepis* (7, 16), *Dianthus* (41), the New World diploid species of *Gossypium* (9, 15, 135, 159, 160), *Lycopersicum* (55), *Solanum* (114, 115), *Vaccinium* (31) and *Veronica* (48). In *Antirrhinum, Aster, Crepis, Lycopersicum* and *Vaccinium* evidence either from artificially raised F_2 populations or from hybrid swarms existing in nature indicates that no barrier to gene exchange exists between the parents of the hybrids concerned, once these are brought together. In nature they are kept apart by geographic or ecological isolation. Under the species definitions of Dobzhansky and of Huxley, these entities would be considered distinct species; under that of Clausen, Keck and Hiesey they would not. If one applied the principles of the latter authors to the evidence of Darrow and Camp (31) on *Vaccinium*, one would be forced to conclude that

the entire subgenus *Cyanococcus* consists of but three species, which
would be differentiated from each other not on morphological char-
acteristics, but on the basis of chromosome number, the first being
diploid, the second tetraploid, and the third hexaploid. This shows
that in a number of genera fertile, cytologically normal hybrids
between recognized species are the rule, and that to apply to such
groups the strictly genetic criteria of species differentiation would
make necessary a very drastic revision of present classifications.

SPECIES HYBRIDS WITH SLIGHT IRREGULARITIES OF MEIOSIS

In spite of this difficult situation in the genera mentioned above,
the statement of Sax still holds good, that "irregular meiotic di-
visions and pollen sterility are characteristic features of most
species-hybrids". Nevertheless, the great majority of F₁ hybrids
produced in the last ten years of which the parents are closely
related species show relatively few meiotic irregularities, and at
least in a large proportion of their sporocytes the chromosomes
are all paired at diakinesis and metaphase. In addition to the four
genera cited by Sax, normal or only slightly irregular meiosis in
partially or completely sterile hybrids has been found in *Apocynum*
(2), *Ceanothus* (81, 82), *Collinsia* (52), *Crepis* (6, 7, 61) *Galeopsis*
(90), *Ixeris*, subg. *Crepidiastrum* × subg. *Paraixeris* (102, 103,
104), *Lactuca* (162), *Layia* (22), *Lycopersicum* (73), *Papaver*
(39), *Phaseolus* (69, 70) *Populus* (110), *Setaria* (79), *Solanum*
(107, 115, 148), *Taraxacum* (112), *Tradescantia* (3), *Tragopogon*
(163) and *Verbena* (33). The explanation suggested by Sax
(125, 126) for *Campsis*, that the sterility of pollen and egg cells
in these hybrids "might be caused by structural hybridity involving
only small chromosome segments", has been adopted by Müntzing
(93) for both intra- and interspecific hybrids of *Galeopsis*, by
Stephens (143) for *Gossypium* hybrids, and by Dobzhansky (34)
for *Primula floribunda* × *verticillata*. Upcott (154), after a de-
tailed study of meiosis in the latter hybrid and its allopolyploid
derivative, concludes that the sterility of the F₁ is due to unfavorable
gene combinations in the gametes. She does not, however, suggest
a mechanism by which gene combinations leading to sterility in
haploid gametes could when present in a double condition give
fertile diploid gametes. Hence the suggestion of Dobzhansky, that
the fertility of the allopolyploid *P. kewensis* is due to preferential

pairing between the complete homologues of the doubled parental sets, is still the one most easily understood. Such preferential pairing would not be expected unless the chromosomes derived from *P. floribunda* had slightly different gene arrangements from those of *P. verticillata*, so that their affinity for each other, though strong enough to enable them to pair in the diploid, would nevertheless be weaker than that between the structurally identical chromosomes produced by doubling the somatic complement of the diploid F_1 hybrid.

This increasing number of sterile species hybrids with essentially normal meiosis is of more than usual interest. At least some of them must come from parents only recently differentiated from each other, in which the sterility barrier is as yet incomplete. An understanding of the cause or causes for their sterility should tell us much about how interspecific isolating mechanisms come into being, or more briefly, about the origin of species. This is made even more likely by the discovery that within certain species, such as *Galeopsis Tetrahit* (89, 90, 93), *Oryza sativa* (149) and *Bromus carinatus* (140), there exist strains which when crossed produce F_1 hybrids having sterility phenomena exactly comparable in both kind and degree to those of the interspecific hybrids mentioned above. Müntzing (93), after a careful study of several intra- and interspecific hybrids of this type in *Galeopsis*, favors the hypothesis of hybridity for small structural differences in the chromosomes in all of his examples for two reasons: *a*) in most examples, both intra- and interspecific, he found univalent chromosomes in some sporocytes, which would be most easily explained on the basis of slight reduction in structural homology of the parental chromosomes; and *b*) in a few interracial hybrids of the tetraploid *G. Tetrahit*, but never in the parental strains, he found multivalent configurations in occasional sporocytes, in spite of the fact that the total amount of chromosome pairing was less than that in the parental strains. These could be most easily explained as resulting from interchange of small chromosomal segments between the ancestors of the parental races, or from a reduction of the structural homology of chromosomes derived from the same parental diploid species to such an extent that preferential pairing was partly eliminated. Sturtevant (144) has suggested a mechanism of inversion and reinversion of the same chromosome segment by means

of which metaphase pairing would still appear normal, but would result in gametes with probably lethal duplications and deficiencies whenever crossing-over occurred in the reinverted segment. Similar mechanisms can undoubtedly be hypothesized in regard to translocations. Although, therefore, much more evidence is needed, that which we have favors the hypothesis that the sterility of F_1 hybrids with apparently normal meiosis is in most cases due to the existence of structural hybridity for small chromosomal segments. Partial confirmation of this hypothesis has been obtained by examination of pachytene configurations in *Antirrhinum* hybrids (38), but evidence of this sort is still far too scanty. If the hypothesis is correct, then the data on segregation in F_2 of a hybrid between such closely related species as *Galeopsis Tetrahit* and *G. bifida* (90) indicate that several such small structural differences must exist between two groups of populations before they will become effectively isolated from each other genetically, and thus represent distinct species. Sax (125) suggests that a difference in four or five segmental interchanges would result in almost complete sterility in the F_1. In *Galeopsis,* however, the effect of each structural difference must be less than was conceived by Sax, judging from the nature of segregation in F_2.

STRUCTURAL HYBRIDITY AND ITS EFFECTS

The hypothesis that structural hybridity for small chromosome segments, or cryptic structural hybridity, is responsible for the haplontic sterility (*i.e.,* sterility in the haplophase or gametophyte, *cf.* Müntzing, 90) of hybrids with normal sexual structures and apparently normal meiosis is strengthened by the fact that between hybrids of this type and those which have very irregular meiotic divisions there is a whole series of intermediate types, which show gradually increasing degrees of meiotic irregularity. Hybrids frequently are found in which all of the chromosomes become paired in a large proportion of the sporocytes, but in which many other sporocytes contain unpaired univalents, and there are also such irregularities as lagging chromosomes, chromatid bridges and acentric chromosome fragments. These have been reported in *Aegilops* (63, 64, 127), *Allium* (36, 37, 83), *Crepis* (6), *Godetia* (51, 53), *Gossypium* (1, 9, 131, 134), *Lactuca* (162), *Layia* (22), *Lilium* (118, 119), *Mirabilis* (129) and *Paeonia* (27, 137).

The effect on chromosome behavior at meiosis of structural hybridity for inversions has been most carefully studied in the now classic work of Richardson (119) on *Lilium martagon album* × *L. Hansonii*. Depending on the number and character of the chiasmata or cross-overs within any inverted segment, the configuration seen at first metaphase or anaphase may consist of a single chromatid bridge and a chromatid fragment, of a double chromatid bridge and two chromatid fragments, of a loop and a fragment, or of two loops and two fragments. The two latter configurations, which result from crossing over proximal to as well as within the inverted segment, give chromatid bridges at second anaphase. The interpretation of these configurations is further discussed by Darlington (28, *265–274*). Emsweller and Jones (37) have shown how the segregation of the products of these and other configurations resulting from the pairing of structurally dissimilar chromosomes can produce gametes with new chromosomal types. By far the greater number of such gametes will be deficient for vital sections of chromosome material, but it is possible that viable types of chromosomes may occasionally be formed in this manner. Sveshnikova (145, 146) has reported an instance of this nature in progeny of *Vicia sativa* × *amphicarpha,* but the new chromosome is apparently viable only in the heterozygous condition, and lethal when homozygous. The significance of bridges and fragments as causes of sterility in species hybrids is considerably reduced by the discovery that these configurations, and hence structural hybridity for inversions, are very common in some fertile species, as in *Paeonia* (137) and *Paris* (40).

The presence of rings, chains or other associations of more than two chromosomes, indicating in diploid hybrids structural hybridity for interchanges of relatively large chromosomal segments, has been found in an increasing number of diploid hybrids, both intra- and interspecific (5, 11, 12, 13, 14, 24, 25, 26, 29, 116, 139). The situation in the genus *Oenothera,* in which this type of structural hybridity is most strongly developed, has been ably reviewed by Cleland (24, 25, 26). Here the cytogenetic and morphological picture, although showing certain regularities, is so complex that Cleland is forced to conclude that (26, 27) "the North American euoenotheras do not show a clear-cut segregation into readily recognizable species." In *Datura* the number of interchange types is

much smaller, and the balanced lethal mechanism characteristic of
Oenothera is absent, so that rings of 4, 6, or 8 chromosomes, or
plants with 12 bivalents, are most commonly found in interracial
or interspecific hybrids (11, 12, 13, 14). Each type of chro-
mosomal arrangement has been designated a "prime type". In *D.
stramonium*, ten such types are known, each differing from its
closest relative by a single interchange, and giving a ring of four
plus ten pairs in hybrids with it. Hybrids between more distantly
related prime types may give two rings of four, a ring of six, or
more complex configurations. In *D. quercifolia* one of the three
types known is the same as one of the prime types (Pt. (2+3)) of
D. stramonium, and gives twelve pairs in interspecific hybrids (14).
However, if other prime types of *D. stramonium* or *D. quercifolia*
are used, the *quercifolia-stramonium* hybrids have rings of various
sizes, depending on the prime types used as parent. In all other
interspecific hybrids of *Datura*, rings of various sizes are found,
but no configurations are more complex than those known in certain
hybrids between prime types within *D. stramonium* (14). Hence
the type of structural hybridity which has led to the formation of
prime types is of little or no importance in the differentiation of
species in *Datura*. *Paeonia californica* and *P. Brownii* are inter-
mediate between the *Datura* type of interchange pattern and the
Oenothera type (139, 158). In both these species of *Paeonia*,
forms with the haploid number of five bivalents are known, but
other forms may have rings of four, six, eight or ten, or various
combinations of rings. The type of ring may be constant in all or
nearly all of the plants in a colony, or it may vary greatly from
plant to plant. Nevertheless, the geographic distribution of the
various types of configurations has a definite pattern, which may
reflect in some way the evolutionary history of the species. In
Godetia Whitneyi, Hiorth (53) reports the presence of a complex
series of interchange types in a small section of the range of the
species in northwestern California. Elsewhere the segmental ar-
rangement of the chromosomes within the species is so uniform
that races from Vancouver Island, the northern limit of the range
of the species, give hybrids having seven bivalents when crossed
with most other races, including those from the north shore of
San Francisco Bay, the southern limit of the species. Since these
races with the most widespread segmental arrangement give hy-

brids with seven pairs when crossed with the related *G. amoena,* the interchange types in races of *G. Whitneyi* from northwestern California are believed by Hiorth to be of recent origin. In this area the populations of *G. Whitneyi,* largely isolated from each other by the nature of the terrain, are evolving a complex series of morphological and cytological races, some of which may be incipient species.

Convincing evidence for the phylogenetic alteration of the basic chromosome number by means of large, unequal reciprocal translocations of chromosomal segments has been obtained in *Vicia* (145, 146) and *Crepis* (150). In the latter genus, a model for this process was created by Gerassinova (42), using reciprocal translocations produced by means of x-rays in *C. tectorum.* Then in the F_1 species hybrid *C. neglecta × fuliginosa* Tobgy (150) found two types of multivalent configurations, involving different groups of chromosomes. Each chromosome could be identified individually by its size and morphology at both mitosis and meiosis, and was given a letter corresponding to those previously used in phylogenetic studies of the genus. The hybrid has seven chromosomes, A, B, C and D from *C. neglecta* ($n = 4$), and A, B and D from *C. fuliginosa* ($n = 3$). The A and D chromosomes of the two species, although appearing as bivalents or univalents in most of the sporocytes of the F_1 hybrid, formed occasional trivalents, and in 10% of the cells were united to form a single quadrivalent. The remaining three chromosomes, B and C of *neglecta,* and B of *fuliginosa,* found a trivalent in 25% of the hybrid sporocytes, indicating that the B chromosome of *fuliginosa* contains segments homologous to portions of both the B and C of *neglecta.* Morphological and distributional evidence both indicate strongly that *C. neglecta* is more primitive and *C. fuliginosa* is derived from it or a closely related ancestor. Furthermore, the C chromosome of *neglecta,* the one not visibly represented in *C. fuliginosa,* was found to be largely heterochromatic. Hence Tobgy makes the very plausible assumption that during the evolution of *C. fuliginosa* the genetically active material of the C chromosome of its *neglecta*-like ancester became translocated to the B. The remaining inert portion of the C, including the centromere, subsequently became lost. Tobgy concludes that Navashin's (98) "dislocation" hypothesis, involving simple translocations, is a less likely one than that of

Darlington (28, *559*) who postulates unequal reciprocal transloca-tions, or segmental interchange, as a mechanism for alteration of the basic number. Sherman (128) has produced evidence that the four-paired *Crepis Kotschyana* evolved in a similar manner from a five-paired ancestor related to *C. foetida.*

Thus the hypothesis advanced by Darlington on cytological grounds and by Babcock on the basis of comparative morphology, that alterations of the basic chromosome number may involve de-crease as well as increase, and are brought about by unequal recip-rocal translocation or segmental interchange, has received striking confirmation from studies of the cytology of species hybrids. This mechanism could account for the phylogenetic reduction of the basic number postulated by Avdulov (4) for certain Gramineae, by Manton (84) for some Cruciferae, and by various other authors. Increase of the basic number by a single pair, such as seems to have taken place in *Biscutella* (84), *Fritillaria* (28, *560*) and *Vicia* (145, 146), may also have resulted in part from unequal segmental interchange, as pointed out by Darlington (28). The greater fre-quency of multivalents in hybrids of *Carex* than in pure species (157) indicates that the extensive aneuploid series of chromosome numbers found in this genus has been formed at least in part by unequal translocations. Wahl (157) made the very plausible sug-gestion that it is the result of the combined action of polyploidy and reciprocal translocation.

HYBRIDS WITH VERY IRREGULAR MEIOSIS

The type of chromosome behavior usually associated with inter-specific hybrids, namely, that involving failure of chromosome pair-ing, lagging of univalents and other gross chromosomal irregulari-ties, has been found in many diploid hybrids, but is not as char-acteristic of them as has often been supposed. In fact, during the past ten years hybrids in which the modal number of sporocytes contain two or more univalents have been described in only eleven genera, while hybrids in which the chromosomes are all paired or in multivalent associations at meiosis have been reported in 41 genera. Hybrids with marked chromosomal irregularities have been reported in *Brassica* (151, 152), *Crepis* (6), *Gossypium* (1, 9, 10, 134, 159, 160), *Lactuca* (162), *Layia* (22, 23), *Madia* (23), *Nicotiana* (35, 43, 44, 68) *Oryza* (117), *Paeonia* (137), *Papaver*

(167) and *Triticum-Aegilops* (63, 64, 65, 127). As might be expected, the parents of these hybrids are as a rule more distantly related to each other, according to accepted taxonomic classifications, than are those species which form hybrids with more regular meiosis. In *Crepis, Gossypium* and *Lactuca* all these hybrids with more irregular meiosis are between species belonging to different sections or subgenera, and this is true of most hybrids of this type in *Layia* and *Triticum-Aegilops*. Of the other two genera listed above in which a considerable number of hybrids is known, namely *Paeonia* and *Nicotiana,* the former is clearly a very old genus, with many of its species probably extinct and with most of the diploid species very sharply distinct from each other. In *Nicotiana* there seem to be many hybrids with irregular meiosis whose parents belong to the same species group (43). Apparently the factors which restrict chromosome pairing have evolved relatively rapidly in this genus compared with those which produce the morphological distinctions between species. In general, however, the statement may be safely made that when a species is crossed with its nearest relatives, the interspecific hybrids usually have more or less regular meiosis, with univalents present in a minority of the sporocytes.

The observation of Sax, that in hybrids with irregular meiosis "there is great variability in the amount of pairing in different pollen mother cells", has held true for all the examples described in the past ten years. Furthermore, much of this variation is controlled by environment. In addition, when a cross between two species has been repeated, using different parental stocks, the results have sometimes been very different. The most striking difference is that between the *Allium cepa* × *fistulosum* hybrid obtained by Levan (74, 76), and those obtained earlier by Emsweller and Jones (36) and Maeda (83). In Levan's hybrid, of which the *fistulosum* parent came from a European botanical garden, only 2% of the sporocytes had the full complement of eight bivalents, while this was true in 72% of the sporocytes in the hybrid of Emsweller and Jones. For the latter, the *fistulosum* parent was a Japanese variety. In a later paper (37) these latter authors state in passing, but without giving date, that different hybrids between *A. cepa* and *A. fistulosum,* using still different parental stocks, differ considerably from each other in fertility and chromosome behavior.

POLYPLOID SPECIES HYBRIDS

When polyploidy is involved, chromosome behavior in species hybrids, as Sax (126) pointed out, is complicated by the fact that pairing may occur partly or entirely between chromosomes contributed by the same parent. A striking example of this is *Ambrosia elatior* × *trifida* (62). In this case both species are polyploids, and the 12 chromosomes in the haploid set of *A. trifida* are recognizably larger than the 18 from *A. elatior*. Although a maximum of 10–12 bivalents may be formed, pairing occurs almost entirely between chromosomes of similar size, and therefore contributed by the same parent. Sometimes this type of autosyndetic pairing is found in hybrids between closely related polyploid species, in which allosyndetic pairing between chromosomes derived from the two parents also takes place. This results in the formation of multivalents and of a cytological picture simulating that found in autopolyploids. An extreme example of this is a triploid hybrid between two diploid species, *Lolium loliaceum* × *rigidum* (60). This apparently resulted from fertilization of an unreduced, diploid gamete of *L. rigidum* by a normal haploid gamete of *L. loliaceum*. In some sporocytes of this triploid F_1 hybrid all of the 21 chromosomes were grouped into seven trivalents and the prophase (pachytene) of meiosis resembled that found in auto-triploids. In some hybrids, like those of *Phleum* (91, 99, 100) and *Bromus* (141), autosyndesis occurs between chromosomes which are rarely associated with each other in the parental species. Two explanations of this phenomenon have been offered. Müntzing and Prakken (94) suggest a "special genotypically controlled tendency to bivalent formation"; Darlington (28, *198*), on the other hand, advanced the hypothesis of differential affinity. This is based on the fact that chromosomes pair not as units, but segment by segment. If a nucleus, as in an interspecific hybrid or a haploid, contains chromosomes which have certain larger or smaller segments in common, these segments will pair and form bivalents, in spite of the fact that these same chromosomes contain dissimilar segments that remain unpaired. If, on the other hand, each chromosome in the nucleus has a homologue to which it is similar throughout its length, as well as one with which it has only some segments in common, the chromosomes will show preferential pairing and will be associated predominantly with their exact homologues. Chromosomes,

therefore, show differential affinity in their pairing relationships. They will pair with their exact homologues if such are present, but if these are absent, any two chromosomes that possess in common large or small homologous segments may form bivalents. Skirm (133) obtained good evidence that this occurs in *Tradescantia*. From a single diploid F_1 interspecific hybrid plant he obtained two types of tetraploid progeny, one of which formed quadrivalents with the frequency typical for autopolyploids, and the other of which formed only bivalents. In the first type, which presumably resulted from fusion of an unreduced gamete, no two chromosomes were exactly identical, and therefore, no preferential pairing was found. In the second type, which must have resulted from somatic doubling of the zygote or young embryo, two identical sets of chromosomes were present, and preferential pairing occurred between them. Whether or not this explanation can be extended to cover all examples of failure of multivalent formation in polyploids must await further evidence.

In some polyploid species, different strains exist which have different degrees of autosyndesis within their haploid sets. A particularly good example is that of the wheat-rye hybrids obtained by Lebedev (71, 72). Autosyndesis of *Triticum aestivum* chromosomes in hybrids with *Secale cereale* may vary from a mode of zero to one of five bivalents, with seven possible in some cells. The *Triticum* strains exhibiting these differences were all from Afghanistan, and relatively closely related to each other; in fact two of them were sister seedlings segregating from the same heterozygous parent. There is no evidence that *Triticum* and *Secale* chromosomes ever pair with each other.

The recent literature on allopolyploid and amphidiploid hybrids has already been reviewed by Goodspeed and Bradley (46). In this connection, however, may be mentioned the newer examples in which one or both of the parental genoms of an allopolyploid have been identified by hybridization with the appropriate diploid species. An outstanding example is that of the tetraploid New World species of cotton, *Gossypium purpurascens, G. barbadense, G. hirsutum, G. Darwinii* and their relatives (8, 15, 134, 135, 142, 143, 159, 160), *Brassica carinata, B. juncea* and *B. Napus* (87, 152), *Setaria Faberii* (77), *Godetia nutans* (51, 53), *Bromus carinatus* (140), *B. arizonicus* (141), *Nicotiana Arentsii* (44) *and*

Madia citrigracilis (23). Further new information on the analysis and synthesis of polyploids is found in the excellent review of the more important literature by Clausen, Keck, and Hiesey (23).

In many allopolyploids, such as *Triticum-Aegilops* (63, 64) and certain annual species of *Bromus* (66), the component genoms of related species are partly homologous with each other. In such cases the exact ancestors of the polyploid are hard to identify, and the classification of the genoms into distinct types is difficult or impossible. Hybrids between two such allopolyploids show complex types of chromosome pairing, like those found in *Agropyron junceum × repens* (106), the various *Triticum × Agropyron* hybrids (105, 109, 111, 113, 123, 155, 156), and the hybrid between sugar cane (*Saccharum* forms) and various species of the genera *Erianthus* and *Sorghum* (58, 88).

THE ORIGIN OF HYBRID STERILITY

Based partly on the evidence from chromosome behavior in diploid species hybrids, many workers (*cf.* 138) now accept a working hypothesis to explain the origin of sterility in F_1 species hybrids. These are believed to originate not through the accumulation of the type of genetic factor responsible for the differences in external morphology and physiology between the groups concerned, *i.e.*, the differences which produce subspecies or races. On the contrary, the separation of species by internal physiological genetic barriers, which are responsible for this sterility, is usually produced by a separate set of genetic factors, which by themselves do not necessarily bring about the visible differences between species. These genetic factors may be separated into two groups. Those of the first group affect the mature F_1 hybrid at the critical stage of the formation of the reproductive organs and the archesporium. They thus produce diplontic sterility (90, *315*), or sterility in the diplophase. Examples are various hybrids of *Paeonia* (124) and *Epilobium* (see 138 for references).

The second and more significant group consists of those types of factors which affect the critical stage of meiosis itself, producing abnormal micro- or megaspores, or cause inviability of the gametophyte or gametes, thereby producing haplontic sterility (90). This group consists of two general types. One type includes genic differences causing upsets of the spindle mechanism, timing rela-

tions and other physiological elements of the meiotic process (17, 18, 30, 59, 67, 75, 78, 147, 153). These produce the genic sterility of Dobzhansky (34). The second and much more widespread type of factors appears to be predominantly if not entirely chromosomal in nature. When the parental species have very different chromosome numbers, as in crosses between diploids and polyploids, or between plants having different degree of polyploidy, the factors for chromosomal hybrid sterility involve largely the irregular distribution of the chromosomes to the poles of the spindle, and the resulting formation of gametes bearing unbalanced chromosome numbers and consequently lethal. If, on the other hand, the parents have the same chromosome numbers and are closely related, the distribution of the chromosomes to the gametes is usually normal or nearly so, and the haplontic sterility is due to inviable conditions within the chromosomes themselves. As explained above, the sterility factors in this case are most likely small structural differences, which are not sufficient to prevent chromosome pairing, but give inviable chromosome combinations through their independent segregation, or inviable new chromosomes through pairing and crossing-over (65, 93, 125, 126, 143). This last type, which can be termed "cryptic structural hybridity", appears to be the most common and significant type of barrier producing genetic isolation between species of higher plants. Although the present evidence suggests that as a rule these different types of sterility factors act independently of each other to a great extent, it is highly probable that some genetic differences between species affect two or more of these different types of sterility.

The existence and partial independence of these different groups of species-isolating factors is indicated by evidence suggesting that they have evolved at different rates in different genera. In *Quercus, Salix, Vaccinium* and other genera in which species with widely different external morphology and physiological adaptations form fertile hybrids, none of these factors has become established. In other genera, *e.g., Galeopsis* (90), *Solanum* sect. *Tuberarium* (115) and *Datura,* many hybrid combinations, even between species that are not very different in external morphology, fail because of incompatibility, and all the hybrids that can be produced have good pairing of the parental chromosomes, and usually at least partial fertility. Here the first group of isolating factors, those affecting

compatibility, appear to have evolved more rapidly than the others. Still other genera, like *Crepis, Layia* and *Gossypium,* have a more or less normal pattern of species differentiation, in which the degree of morphological differentiation, of incompatibility, and of hybrid sterility of the chromosomal type are roughly correlated with each other. In these genera the divergence in morphological characteristics and in the various types of genetic isolating factors appears to have progressed at about the same rate. Then there are genera like *Triticum-Aegilops* in which the majority of species differ strikingly from each other in the external morphology of their chromosomes, and nearly all of the species hybrids, although most of them can be made without great difficulty, are characterized by extreme irregularity in chromosome pairing. In such genera the repatterning of the chromosomes through the accumulation of small structural differences appears to have gone on with exceptional rapidity.

The causes of species divergence with respect to these morphologically neutral genetic isolating factors are not yet clear, but some definite hypotheses concerning them have been formulated. The difficulty of accounting for this divergence through natural selection was already recognized by Darwin (32, ch. 9), and is considered practically insurmountable by most recent authors. There are, however, two evolutionary processes now recognized which are independent of natural selection and which could account at least in part for the divergence. These are the effects of sampling in small populations (drift, random fixation) and hybridization between preëxisting species. The effects of sampling could produce in a small isolated population the random fixation of one or more factors which would not affect intercrossing or F_1 fertility in crosses between members of this population, but would produce such effects in crosses with members of other populations. Earlier in this review evidence has been cited to show the occasional presence of partial incompatibility or sterility in crosses within species. Hence it is not unreasonable to assume that if a large species population should because of a change of environment become segregated into several small geographically isolated ones, some of these segregant populations would develop barriers of genetic isolation separating them from the rest. If, then, such a genetically isolated population should increase again, either through the reversal of the

environmental change or through the appearance in the population of favorable mutations, it would form a species morphologically similar to but genetically distinct from the other populations with which it was formerly united. The differentiation of species in this manner by means of passage through a "bottleneck" or series of "bottlenecks" has been postulated by Wright (165). The second method was suggested by the writer (138). If two species differ by a large number of genetic isolation factors, but nevertheless produce an F_1 hybrid which is partly fertile, new recombinations of these factors should occasionally give in later generations types fully fertile but producing partly sterile hybrids in crosses back to both parents. This result has recently been obtained on a large scale by Lamprecht (69, 70) from the hybrid *Phaseolus vulgaris × multiflorus*. In some of these genetically isolated hybrid segregates, greater isolation could result from the occurrence and fixation of additional isolating factors. In the writer's opinion there would be no difficulty in accounting for all genetically isolated plant species as resulting either from passage through a previous bottleneck or from segregation out of partially sterile hybrids between preëxisting species. The species differences in respect to external morphology and physiological adaptation to the environment would in this case be mostly an accentuation of previous subspecies or specific differences. According to this hypothesis, therefore, speciation comes about either through hybridization between preëxisting species or through superposition of genetic isolating factors upon a pattern of intraspecific differentiation into races or subspecies, as postulated by Mayr (85, *160*). Such an hypothesis appears as the natural outcome of our present knowledge of the cytogenetics of species hybrids.

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AUXIN, THE PLANT–GROWTH HORMONE. II[1]

F. W. WENT
California Institute of Technology

I. This continuation of the review on auxin in volume 1 of this journal is necessarily incomplete, for more than 500 papers have appeared on the subject in the last ten years, and space does not permit a more extended treatment. Only a few trends in the field will be enumerated, and whenever a number of papers on the subject have appeared in close sequence, usually only one or a few of them are listed, the choice being more or less arbitrary; availability of them in America, and sometimes priority or completeness in their exposition, have been used as criteria for selection. More stress is laid in this supplement on the theoretically important points than on the practical ones, since another review in this journal by Zimmerman on growth hormones has been announced; Zimmerman is one of the chief exponents of less theoretical treatment of the plant hormone field (38). Detailed information can be found elsewhere (8, 9, 31, 49, 56, 74, 84, 89), and the same subdivision will be followed here as in the original review, although the emphasis on subjects has shifted very much.

II. The Avena method is still the principal method of quantitative analysis of auxins. A modification of it (61), the deseeded Avena test, is more sensitive to low concentrations and has been accepted as the standard test in some laboratories. Other qualitative and semiquantitative tests have been described in great number. Only the "green tissue tests" (38) and the pea test (76, 80) need be mentioned. Also, new auxin units have been introduced, W.A.E. of Boysen Jensen (9) and T.D.C. of Avery *et al.* (2), but they have exactly the same disadvantage as the A.E. of Kögl. They all depend on the relative sensitivity of *Avena* coleoptiles to auxin, which sensitivity varies with time of day, season, locality and laboratory. The only more or less absolute unit, although not ideal, is van Overbeek's (53) indoleacetic acid equivalent. It gives the amount of indoleacetic acid inducing the same curvature in the Avena test as the tested sample.

III. Chemical isolation and identification of indoleacetic acid

from vascular plants has been accomplished (5, 32). This makes it necessary to use the term "auxin" as a generic name for all substances, produced by a plant as growth hormones or as correlation carriers, which give a response in the Avena test.

Concerning the chemistry of auxin a and b, only a few additional facts have been published (43), showing that inactivation of the auxin a and b lactons occurs in ultraviolet light. The question whether auxin inside the plant is largely inactivated by visible light is still unsettled (45, 50, 69).

Soon after the discovery of the effectiveness of indoleacetic acid as an auxin (42), a deluge of articles appeared, all publishing the growth-promoting properties of other substances (33, 35, 44, 93). Since these substances vary in activity from highly active to almost inactive, and since they have a fairly wide range of structure, the conclusion was drawn that their effect on plants is an unspecific stimulation (1, 21, 38). Three groups of facts, however, speak against such an explanation of auxin acting as a stimulant.

First, it could be established (41) that all active substances have certain structural characteristics in common: an unsaturated ring with side chain of at least two carbon atoms adjacent to double bond, a carboxyl group in the side chain, and a definite space relationship between carboxyl group and double bond.

Secondly, closer analysis showed that the apparent activity of auxin-like substances is reduced by side-reactions and by secondary properties of those substances which have nothing to do with the growth reaction (6, 72). When only the number of molecules actually partaking in the growth reaction is considered, the molar activity of all auxin-like substances is the same (82, 87).

The third group of facts indicating that the effect of auxin on the growing cell more closely resembles a chemical reaction than an unspecific stimulation will be mentioned under VII.

IV. Production of auxin as a function of the growing condition of the plant has been extensively investigated, and very clear correlations between growth rate of shoots during their grand period of growth and auxin production by the stem tip have been found (3, 89, 94). Nutrient deficiencies, especially of N and Zn, decreased auxin production (4, 63).

Auxin has been found in three forms in the plant (for further discussion see (56, 86)), namely,

Inactive in the Avena test:

    a) Bound auxin: extractable with organic solvents; liberated after chymotrypsin treatment (76).

    b) Precursor: activated in the plant by enzymatic action in stem tip (55); activated *in vitro* by alkaline hydrolysis (2, 5, 32).

Active in the Avena test:

    Free moving auxin; diffuses out of plant into water or agar.

These forms have different functions in the plant. The precursor is the source of all, being transformed into free moving auxin which regulates correlation phenomena, such as photo- and geotropism (85). Part of this free moving auxin becomes bound and then regulates growth.

Many papers have appeared on extraction methods. None is entirely satisfactory or is applicable in all cases. Most troublesome is the phenomenon that many materials, notably leaves, stems and fruits, when extracted with organic solvents, continue to give off auxin for many months (29). Only one conclusion emerges from all this work: in each case the best extraction method has to be found empirically and has to be adjusted to the problem for which data are needed.

Other difficulties may be encountered in analyses of the auxin content of tissues. Inhibitors, for instance, may be present (67) which mask or reduce the actual auxin concentration in the extract (25), or the auxin may be destroyed before or during extraction (52). Dwarf genes have been held responsible for excessive auxin destruction inside the plant.

V. Auxin transport was confirmed as polar inside all living tissues, except when pharmacological doses were applied (90). When, under exceptional conditions, auxin reaches the transpiration stream, it moves with this stream against its normal basipetal direction of transport (36). As soon as this auxin has moved back into living tissues it moves polarly again (62). Polar auxin transport may occur simultaneously with non-polar ion transport (83). Inversion of morphological polarity may be followed by establishment of an inverted polarity of auxin translocation (85).

Much work was done to correlate the polarity of auxin transport with electrical polarity, with little success, however (12).

Protoplasmic streaming was speeded by auxin (18) and was

found to be a function of the concentration of oxygen, sugar, auxin and malic acid applied (71).

VI. In several cases the interrelationships were investigated between auxin and other factors in speeding up stem elongation. Special emphasis was laid on the interaction between auxin and sugar (59) and between auxin and caulocaline (77, 81).

VII. The assumption that auxin is a coenzyme required for some process essential in growth, was greatly strengthened by recent work (14). It was found that about 10% of the normal respiration of *Avena* coleoptile is auxin-controlled, and that this respiration is essential for growth. The auxin might play the rôle of coenzyme in a dicarboxylic acid dehydrogenase in this respiration. This would take auxin completely out of the classification of "stimulant" and make it a specific component of a known chemical reaction. It explains the quantitative relationships between auxin and growth, its specificity and its indispensability.

Further study of the structure of the growing cell wall gave rise to more elaborate theoretical considerations concerning the action of auxin (7, 17, 58).

In addition to the direct and indirect action of auxin on the cell wall, a few other effects of auxin have been invoked to explain their effect on growth. To these belong the effect of auxin on active water uptake by the cell (13, 64), their effect in the so-called preparatory reaction (80) and their effect on the translocation of other growth factors (78).

VIII. The earlier controversies on the effects of auxin on the growth of roots and auxin production by roots have now subsided. Only at extremely low concentrations is root growth accelerated by auxin, but above 10^{-10} molar it is inhibited (24). Very little auxin is synthesized by roots in their tips; most of it must be derived from the top (54, 57).

IX. The number of processes in which auxin is known to take part, besides stem elongation, has greatly increased. It is among these most recently recognized processes that the most practical applications of auxins have developed:

a) Bud inhibition. All investigators agree now with Thimann and Skoog that auxin coming from the growing stem tip is the correlation carrier which prevents development of lateral buds. But there is utter disagreement as to the way in which auxin in-

hibits. The literature on this subject has been summarized (73), and actual auxin analyses of inhibited and non-inhibited buds and adjoining stems have been published (20, 51). Sprouting of dormant buds in potatoes and deciduous trees can be delayed with auxin sprays (30).

b) Initiation of roots on stems is also caused by auxins. This discovery has led to one of the most important practical applications of auxins, the rooting of cuttings with auxins and auxin-like substances. Consequently the number of papers on this auxin effect exceeds that on all other effects, mostly concerning the best method of treatment and the most active compound (extensive lists of plants which can be rooted by auxins are to be found in 48 and 49). A variety of methods of application has been used: at apical end of cutting (15); solutions of about 0.01% at basal end of cutting (37); dipping of base in auxin dispersed in talc (26); dipping in alcoholic solutions of auxins (39). Age of plant from which cutting is taken (74), pretreatment of cutting (16), use of additional root growth stimulators (70, 87) and many other factors influence the rooting response.

Theoretical considerations on the mode of auxin action in the rooting response are found in many articles (e.g., 16, 77, 78).

c) Much work has been done on the anatomical changes in tissues caused by application of auxin; swelling of cells, cell division, dedifferentiation and redifferentiation of cells and tissues are commonly observed (34, 60).

d) Another important application of auxin is based on experiments (22, 27) showing that auxin treatment of unfertilized ovaries or styles of many plants led to the development of parthenocarpic fruits of normal size. It seems that under normal conditions of fruit growth, ovules and young seeds furnish the auxin necessary for growth, which has to be applied externally when no seeds are formed (28). Commercial production of seedless fruits is possible in this way (40).

Application of auxins at a later stage of fruit development may check fruit drop. This is used commercially in some apple varieties to prevent pre-harvest drop (23).

e) Among the cases in which auxin increases cell division, cambial activity is the most outstanding. Application of auxin to stems causes cambial activity over some distance below the point of application (65, 66).

f) Much work has been done on the production and anatomy of abnormal tissues induced by (unphysiologically) high auxin concentrations. In many plants structures closely resembling crown gall can be induced by auxins (11, 46, 47).

g) Many morphological changes of various kinds have been described after application of a variety of substances, some of them resembling auxins in structure (90, 91).

h) Addition of auxin to the nutrient solution may partly overcome zinc and boron deficiencies (19, 63). This effect may be related to the effect of auxin on root activity in general (64).

i) A large number of papers has appeared describing the effects of auxin applied to roots or seeds. Many authors claim that such treatments cause after-effects in the form of increased or decreased growth, earlier flowering, better fruit set, *etc.* But others failed to find after-effects. The described effects are probably of complex origin, and more has to be found concerning the exact conditions under which the growth stimulations are reproducible, before general conclusions can be drawn. It is perhaps suggestive that fertility of soil is correlated with its auxin content (68).

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LIGHT AS AN ECOLOGICAL FACTOR AND ITS MEASUREMENT. II[1]

HARDY L. SHIRLEY

New York State College of Forestry[2]

INTRODUCTION

Refinement in knowledge and new scientific discoveries follow closely behind the introduction of new scientific instruments and new scientific techniques. And in no phase of botany has this been more evident than in the general field of plants in relation to light. Of outstanding importance has been the general availability of cheap reliable light-measuring devices that use as their sensitive element the photoelectric cell. Equally important have been the advances made in growing plants in controlled environment through the use of newly developed artificial sources of light, particularly the neon and sodium vapor lamps and the filters that transmit definite ranges of wave lengths. As a result recent workers have been able to surpass their predecessors in the accuracy of light measurement and in the precision of light control. And through advances in experimental design and statistical analysis developed by Professor R. A. Fisher and his American disciples (55, 156) they have learned how to analyze the separate and combined effects of varying several factors simultaneously in the same experiment. All three of these developments appeared before 1935 when the original paper under this title was published (148), but had not yet been put to widespread use in botanical research.

Just as the trends in research might have been predicted ten years ago on the basis of new light sources, new light measurement devices and new experimental design and analyses, so today we may predict that advances in understanding the relationship of light to plants during the coming ten years will result from a more widespread use of fluorescent light sources and the introduction of heavy carbon as an indicator element in photosynthesis. We may expect further refinement of the mathematical relationships between total available light and plant growth as a result of the increased general availability of light recorders.

Considerable confusion has been introduced into the literature by the tendency of many investigators to attribute to light certain responses resulting mainly from other causes. Students of plant life must bear ever uppermost in mind the fact that light, being necessary for the photosynthetic process, cannot be varied widely without introducing the factor of malnutrition or starvation. Plants starved for the products of photosynthesis can no more react normally to other conditions than those starved for nitrogen, for phosphorus or for calcium. Likewise, plants starved for mineral elements or provided an inadequate moisture supply inevitably respond differently to light than do normal plants. With sufficient time for adjustment, plants, like all other living organisms, show wide adaptability to different light conditions. But when adjusted to one they cannot quickly respond to a different light condition. For instance, if adjusted to living under very low light intensity, plants may be injured or even killed by abrupt exposure to a high intensity to which, given ample time, they could have become adapted.

Although in the discussion to follow, the various effects of light on plants will in most cases be considered singly, it must be kept in mind that such effects are by no means independent of other environmental conditions.

In preparing the current review, the writer has omitted several important topics if they were covered adequately in his earlier reviews (47, 148) or in the excellent reviews by other workers (26, 27, 62). Material presented in foreign reviews (146) has been covered in part.

LIGHT MEASUREMENT

Excellent reviews of light measurement technique have appeared in both English and German literature since 1935 (45, 47, 58, 96, 108). These reviews describe the various effects produced by light that are susceptible of measurement and the instruments that have been devised to measure these effects. Tests have been made on shape of absorbing surfaces by Miller (102) and Wallace (173). Flat horizontal surfaces can absorb only the vertical component of solar radiation and hence give lower values than spherical absorbers except when the sun is in the zenith. The ecologist concerned with the light received by a single isolated tree or shrub might

prefer to use the spherical absorber, but those studying the light received by the vegetation of a definite area will find the horizontal absorber satisfactory. The most popular instrument that has come into widespread use since 1935 has been the photoelectric cell in its various forms, particularly the photronic cell with a sensitivity curve approximating that of the human eye (53, 169). Such cells have been built into photographic exposure meters that are highly portable, relatively low in price, and hence available to most ecologists. The exposure meter has a geometric scale, making it particularly useful in measuring light of low intensity. It is not, of course, precise at high intensity.

For many studies, direct reading devices for light measurement need to be supplemented by light recorders and integrators such as used by Wallace (172, 173) and MacLean (91). Sprague and Williams (157) have devised a very ingenious and inexpensive integrating light recorder consisting of a photoelectric cell, condenser, a cold cathode discharge relay tube, a second condenser and a sensitive electrical impulse counter. Sprague reports the cost of these instruments as $12.00 to $25.00 (157, 158). A simple and thoroughly sound instrument for integrating solar radiation over a period of time has been described by Byram and Jemison (31). This consists simply of two pairs of calorimeters, one pair of which receives solar radiation while the other is shielded from it. The radiation is measured directly in gram calories per square centimeter by determining the amount of ice melted by absorbed solar energy.

Certain precautions are necessary to get reliable measurements of light in the forest. With photoelectric cells, care must be taken to avoid over-exposure to intense radiation. Under such conditions the cells solarize, *i.e.*, their sensitivity declines. To avoid this and at the same time to get satisfactory readings of full daylight intensity, flat or spherical opalescent glass screens may be used (172, 173, 183). It is necessary to calibrate each individual screen, and if a plain glass surface is used, it must be exposed at normal incidence to sunlight or allowance made for the reflection from the glass surface with different solar angles. Another way to avoid solarization of photoelectric cells is to use them only to measure the light received from a standard mat surface reflector. The reflector, in so far as possible, should have a perfect diffusing

surface, and as a further precaution the photoelectric cells should be held in such a way as to avoid specular, mirror-like, reflection. Standard high-grade bond paper can be used as such a reflector. When a sheet becomes soiled it should be replaced by a fresh one. Painted boards or white opalescent glass surfaces soon accumulate dirt and even though washed frequently are likely in time to change their reflecting capacity.

A common and important source of error in using direct reading devices, either in the open or in the forest, is to allow light reflected from the clothing of the observer or other objects to reach the sensitive elements (187). Reflected light can be particularly disturbing should the reflector be in a sun fleck and the instrument in complete shade. Smith (155) reported that measurements taken with the photographic exposure meter between 9:00 a.m. and 3:00 p.m., used in conjunction with a board coated with aluminum paint, failed to show up differences resulting from thinning; whereas Walton (176), using technique similar to that described by Smith, found that results of thinning showed up quite satisfactorily. Walton and other observers report much more consistent results when the sky is overcast, thereby avoiding the disturbing influence of sun flecks. But readings made in the forest on cloudy days should be compared with simultaneous readings in the open, as absorption of light by clouds varies widely from moment to moment. Rarely is the sky uniformly overcast for any appreciable length of time. Inasmuch as sun flecks may play a very important role in the life of plants growing beneath forest canopies, sufficient widely distributed measurements should be made in any one habitat so as to sample adequately both complete shade and sun flecks. A still better system was devised by Nägeli (110). He took a great number of readings at various points and at various times of day. Light intensity of the habitat was then expressed in a frequency curve.

Ecologists for the most part have discarded photochemical photometers such as the Wiesner and Clements types. There are, however, situations in which the photographic photometers can be successfully used. Matheson (94) made comparative measurements with a Livingston radio atmometer, solar radiation thermometers, Weston photronic cells and Eder-Hecht photographic photometers. He found the first two both to give unreliable mea-

surements when checked against the Callendar pyrheliometer, whereas the last two, the photronic cell and the photometer, gave satisfactory readings provided their limitations (selective sensitivity to different wave lengths and tendency on the part of the photronic cells to solarize when exposed to high intensity solar radiation) were taken into consideration.

Good progress has been made during the past ten years in building more accurate filters for measuring specific spectral regions (16). Such filters may be obtained commercially from the manufacturers of signal glass and photographic filters, or may be made up by individual workers by tinting cellophane or gelatin with various solutions. The need for precise filters is perhaps much greater in physiological than in ecological studies. Nevertheless, a great many investigators have used such filters in measuring light in the forest and beneath various depths of water in lakes and oceans. In using filters with photoelectric cells, the worker must allow for the selective sensitivity of the photoelectric cell as well as the selective transmission of the filter.

LIGHT CLIMATE

The ecologist studying light conditions in natural habitats today not only has better instruments for measuring light than he had in 1935; he also has at his disposal long-time continuous records of light conditions measured at various stations in North America and Europe. Hand (71) has published tables and isopleths that show the light intensity for each hour of the day and each day of the month at 20 North American stations. Steinhauser (162) and Maurain (96) present similar but less complete information for Europe, giving particular emphasis to the influence of altitude. Byram and Jemison (31) show how the light received at any open station can be approximated by direct computations. Their computations must, of course, be corrected for cloudiness.

Variations in the daily period of illumination with time of year and latitude is one specific aspect of light climate. Though the effect of photoperiod on plants has been treated elsewhere (62), the threshold value of light intensity necessary to produce the photoperiodic response is worthy of note. Several investigators (15, 178, 185) have shown that light of one foot candle, or even slightly less, may be adequate either to induce or inhibit flower formation.

But this occurs only when such light is used to supplement daylight or artificial light of intensities adequate for growth and reserve food accumulation (179). Using one foot candle as a minimum, Greulach (67) has found the length of the photo-periodically effective twilight for north central United States to vary from 11 to 28 minutes with a mean of about 19 minutes. It is significant that one foot candle is only slightly above the intensity of bright moonlight.

Ball (17) found that the red end of the spectrum was far more efficient than the yellow, green or blue in preventing flowering of *Turnera ulmifolia* by illumination at night. This is of rather striking importance, since the low light intensities of early morning and late evening are rich in the red region. The above effect is in direct contrast to ordinary phototropic growth responses, which are stimulated much more by the blue than by the red end of the spectrum.

Several workers (86, 95, 113) have reported that light from street lamps is often of sufficient intensity to cause shade trees growing nearby to retain their leaves in autumn several weeks longer than trees of the same species somewhat more distant from the lamp.

The long arctic summer days are of significance in the growth of plants, both because of the relatively long photosynthetic period each day and because of the photoperiodic response. Both of these influences can, however, be overemphasized. Albright (2), for instance, has pointed out that the tomato, which is adversely affected by a 24-hour day when a portion of that light is supplied from incandescent lamps, thrives quite satisfactorily when exposed to the natural light of 24-hour arctic days. The difference in this case probably is due to the fact that the light of the arctic day, though richer in red than normal daylight, is far lower in infrared than light from an incandescent filament lamp. Arthur and Harvill (9) have shown that the injurious effects of 24-hour illumination to tomato plants can be lessened by decreasing the amount of infrared or by increasing the proportion of either red or blue light in the total artificial light to which the plants are exposed.

Razumov and Smirnova (131) emphasize that the effectiveness of the arctic night light is greatly diminished because of low temperature. Most of the daily photosynthetic activity occurred, he

found, during the day when the sun was relatively high and the temperature warm. On the other hand, Freeland (60) and others have shown that certain conifers, at least, can carry on photosynthesis at a sufficiently rapid rate to more than offset respiration at temperatures of 40° to 50° F., and therefore presumably benefit from light of the arctic night. Printz (130) reports photosynthesis at −2° C. in *Picea excelsa* and *Pinus silvestris*.

Light intensities in the tropics, though normally higher than in temperate regions, are not, in Veth's (171) opinion, so much responsible for the luxuriant growth as the 12-month growing season. He states that growth increases rapidly up to about 20% to 30% of full tropical sunlight, but decreases slightly as intensities increase above 50%. In tropical regions having a minimum of cloudiness, plants thrive on one-fifth to one-twelfth of full light intensity. At greater intensities respiration due to the higher temperatures more than offsets the benefits from increased light. This is the reason why many tropical plants thrive best in shade.

Various workers have reported that light at high altitudes contains an excess of ultraviolet and for that reason is injurious to plants. In fact, some believe that the upper altitudinal limits of species are determined by their relative tolerance to ultraviolet (39). It may be significant to note here that light, particularly ultraviolet, causes a 30% to 50% increase in respiration rate of plants previously conditioned by holding them in darkness for periods up to 70 hours (64, 65).

Solar ultraviolet intensity and its ratio to daylight fluctuate with the intensity of sun spots (12), but it is not known to what extent such fluctuations in ultraviolet radiation affect green plants or animals. It is known that plants can be easily injured by exposure to ultraviolet of wave lengths shorter than that found in sunlight, but it is less certain whether the high intensity ultraviolet encountered at high altitudes and in the tropics is in itself injurious. Also, it has not been clearly demonstrated that ultraviolet is at all necessary for the development of plants (51, 124, 126, 127).

The modifications in light intensity caused by atmospheric turbidity, vegetative canopies and clear or turbid water are more local in action and hence have been referred to as producing a micro-light climate. Turbidity, whether due to haze, smoke or ash in the atmosphere, or silt and clay in water, acts selectively in that it

scatters the short blue rays to a greater extent than the long red and infrared; hence in cloudy weather or within turbid water the light has a higher proportion of red and infrared (33, 72). To a lesser degree a vegetative canopy acts similarly, but the changes in quality caused by scattering or refraction are largely offset by differential transmission and reflection, and by changes in the proportion of direct sunlight to skylight caused by the canopy.

Measurements made beneath forest canopies during the past ten years tend to confirm the conclusion reached in the previous review (148) that changes in light quality resulting from passage through a leafy canopy are of minor ecologic significance (14, 32, 101). This is further borne out by measurements of the light transmitted through leaves and that reflected from them. Compared with herbaceous leaves, those of forest trees have relatively low transmission and relatively low reflection (13). Leaves tend to absorb blue to a greater extent than red, and red more than green. A great deal of blue light from the sky penetrates the canopy, compensating in part for that absorbed by the leaves. Atkins, Poole and Stanbury (14) report that green canopies transmit only 10% more green than blue and 15% more green than red. Sauberer (138, 139) reports measurements of the spectral composition of light that had penetrated one, two, three and four leaves, and of that reflected one to four times from leaves. Foliage that transmitted 3% to 20% of the light through the first leaf allowed only about 1% through the second, and a negligible amount through the third and fourth leaves. Infrared light at 770 mμ reflected four times from leaves was 40%, 16%, 6% and 3%, respectively, and green at 540 mμ 6%, .31%, .0017% and .00095% of incident light. As these measurements indicate, leaves reflect (116) and also transmit (46, 49) relatively large amounts of near infrared, but energy in this region is not directly useful in photosynthesis.

Seybold and Egle (147) evidently believe that leafy canopies do cause changes in light quality of ecological significance. They have made extensive studies of the optical properties of leaves and of leaf pigments. Seybold classifies forest floor species as sun plants—those that appear before the deciduous leaves have unfolded; red-green shade plants—those appearing when the leaves are only partially developed; and green shade plants—those appearing as summer advances. The ratio of chlorophyll A to chloro-

phyll B for the three classes of plants was found to be, respectively, 4.36, 3.01 and 2.60. The ratio of xanthophyll to carotin was 3.6, 5.5 and 5.3 respectively. Corresponding ratios for immersed and submerged water plants were for chlorophyll A to B, respectively, 4.4 and 2.3, and for xanthophyll to carotin, 4.0 and 5.7. The ratios of total chlorophyll to total carotinoids varied only slightly. These changes in the relative concentration of the several photosynthetic pigments are assumed by Seybold to be adaptations to light conditions. The actual significance of this still remains to be demonstrated. Further evidence that minor changes in light quality are of slight ecological significance will be presented later under a description of studies of photosynthesis in natural plant habitats.

Many workers have reported light measurements beneath forest canopies during the past ten years. These show the light beneath the leafless hardwood canopies to be approximately 55%, and to decrease progressively as the leaves unfold until the minimum value, varying from 1% to 5%, is attained (34, 165, 175, 186). Intensities beneath pine canopies have been found by Shirley (152) to vary with age and density of stand. Minimum values for jack pine, 15%, occurred at 30 to 60 years of age. Natural red pine canopies of various ages showed the following intensities: 25–35 years, 7%; 55–65 years, 11%; 90–110 years, 16%; 200 years and over, 44%. Intensities beneath tropical canopies may be reduced to between .2% and 1% of full sunlight (32, 52, 119). Even free standing trees such as occur in apple orchards do not have all their branches or all their leaves exposed to adequate light intensity for maximum photosynthesis (35). Schmidt (141) measured light intensities of 24% to 36% beneath apple trees. Likewise, light intensity available to plants growing near buildings or along the border of forest stands is far greater along the south, east and west than along the north border, and these differences in light significantly affect air temperature, transpiration and assimilation (53a).

Light penetration into clear fresh and salt water follows very definite laws. Penetration through turbid water, on the other hand, is more difficult to calculate because turbidity varies so much. Alvik (4) reports absorption of 20% per meter in clear salt water, and absorption of 65% per meter in brown fresh water. Clarke (36) reports that the water south of Bermuda, the clearest sea water known, shows no change in transparency down to a depth

of 133 meters. Measurements by Utterback, Phifer and Robinson (170) in Crater Lake, Oregon, gave light intensities of 11.5 foot candles at 120 meters depth, at a time when the intensity at the surface was 9,000 foot candles. The extinction coefficient for red light was .311 per meter, for green .060 per meter. For blue light there was a change in coefficient with depth from .031, for the first 40 meters, to .035 from the 40th to the 70th meter.

Croxton *et al.* (42) and Egle (49) have measured light transmission through snow. Croxton showed that a negligible amount penetrated eight inches of snow. Egle found transmission through three, five and ten mm. of snow to be 35%, 20% and 5%, respectively. It is obvious, therefore, that plants covered with as much as 20 mm. of snow receive insufficient light for photosynthesis, assuming that temperatures are adequate for this process.

A special case of micro light-climate is that of the exposure of chloroplasts as determined by leaf structure, particularly in thick-leaved succulents. Schanderl (140) and Sauberer (138, 139) have shown that such plants have special means by which the light is transmitted into the leaves to reach the assimilating tissue. These include light-transmitting tannin cells and sclerenchymatous tissue, and orientation of chloroplast along vertical walls, leaving the interior of the cell free to transmit light to inner layers.

Thin-leaved xerophytes and plants of xeromorphic structure that grow on bogs, heaths and other infertile soils, on the other hand, have in their epidermal layer tissue that strongly diffuses the light, thereby protecting the chloroplasts of the palisade against injuriously high intensities (113A).

LIGHT REQUIREMENTS FOR PHOTOSYNTHESIS

Studies of how the photosynthetic process in various green plants is affected by light of various intensities and wave lengths have helped immensely to clarify understanding of the relationship of plants to the light prevailing in their natural habitats. Determinations of quantum relationships between light absorption at different wave lengths and chemical synthesis by the plants indicate that, within the visible region of the spectrum, photosynthesis is almost directly proportional to the amount of energy absorbed by the photosynthetically active pigments (28, 29, 30, 50, 61, 80). This does not necessarily correspond with the total absorption by leaves, inas-

much as inactive pigments and tissues of the leaves absorb energy that can influence photosynthesis only through increasing leaf temperature. Gabrielsen (61) reports that anthocyanin in the epidermis of leaves of *Corylus maxima* var. *purpurea* reduced the photosynthetic efficiency of the light by 43% compared with that of green leaves of *Corylus maxima*. Because of differential reflection and transmission not all wave lengths are absorbed in proportion to their intensity in the incident light. The red region of the spectrum is the most efficient in photosynthesis in proportion to incident energy. Gabrielsen (61) gives values of .38, .60 and 1.00 for relative photosynthetic efficiency in the blue, green and red regions at wave lengths of 440 mµ, 540 mµ and 650 mµ, respectively. Similar values are reported by many other investigators.

The question naturally arises, can an efficient monochromatic light source be found that will produce satisfactory growth of plants? Arthur and Stewart (10) grew buckwheat plants with continuous illumination from several sources, and report their relative efficiency as follows:

incandescent lamps		1.00
sodium	"	1.41
neon	"	1.20
mercury	"	0.62

It was later discovered, however (9), that certain plants, notably begonia, gardenia, cotton, geranium, buckwheat and snapdragon, tended to degenerate after two or three weeks continuous exposure to sodium vapor lamps, but that this degeneration did not occur if the sodium vapor lamp was supplemented by a mercury vapor lamp. Tomato plants, on the other hand, did poorly when exposed to continuous light from either the sodium or mercury arc or from the two acting together. The hypothesis has been suggested that photosynthesis is more efficient when all regions of the visible spectrum act together than when they act separately. Montfort (107) did not find this to hold for tests on green and brown algae. The concerted opinion of many investigators is that a proper balance of red and blue is necessary for normal development of plants, and that high intensity of a single wave length cannot compensate for inadequate representation of others (40, 44, 136). Johnston (83) states the case well: "Since plants have been growing on the earth for millions of years, it is reasonable to assume that their physiology

is adjusted best to sunlight". Such a conclusion is still a safe work-ing hypothesis for plant ecologists.

Though roughly 50% of solar energy lies within the photo-synthetically active region, less than 1% of that reaching the earth's surface at any point is transformed by vegetation into chemi-cal energy in the form of carbohydrates. For instance, the heat value in calories of the wood, bark, branches, twigs and leaves pro-duced annually by an acre of good white pine in Wisconsin is equiv-alent to only .23% to .5% of the solar energy during the growing season received in an equal area at Madison, Wisconsin (149). Similarly, solar energy utilization by grasses and clover in Bavaria is reported by Noddack and Komor (114) to be .4% to .8%.

But light intensities of 1% or less are far from adequate for maximum photosynthesis. Throughout the range of from 1% to 15% of full sunlight, photosynthesis is almost directly proportional to light intensity, provided other factors essential for rapid photo-synthesis are favorable. Should one or more factors be below optimum, photosynthesis reaches a certain plateau value and re-mains more or less constant with further increase in light intensity. If, on the other hand, all factors are favorable, photosynthesis con-tinues to increase with light intensity until secondary effects set in, such as high respiration caused by high light intensity and high temperature, solarization (the over-accumulation of photosynthate in the leaves and resultant reduction in photosynthesis), water defi-cit causing closing of stomata, or some other internal limitation. Heinicke and Hoffman (79) found that apple leaves failed to achieve maximum photosynthesis at intensities less than 1,200 foot candles. Carnations require approximately 1,500 foot candles, holly 1,940, radishes approximately 760 (153), dogwood, eastern red oak and white oak 3,000, 3,000 and 1,000 foot candles, respectively, loblolly pine 9,000 (87), *Elodea* 12,000 foot candles (64), *Fucus* two-thirds of full sunlight (81), and tomato plants up to the full daylight in-tensity at Grand Rapids, Michigan (128).

Solarization and actual injury occur at very high intensity. Myers and Burr (109) demonstrated that *Chlorella* cells were ir-reparably injured at 40,000 foot candles, and solarization occurred at even lower intensities. Smith (154) published anamorphic curves and derived an equation that expresses the rate of photo-synthesis of water plants in relation to light intensity and carbon

dioxide concentrations. This equation gives an exact relationship except for very low rates of photosynthesis.

The ecologist, and particularly the forester, is concerned with what happens when small seedlings growing on the forest floor are suddenly exposed to high light intensity by logging the overstory trees. Harder's (73) extensive studies with water plants bring out one of the principles involved. His plants were cultivated and tested for photosynthetic activity in light intensities having relative values of 1, 4, 64 and 256. Those cultivated in strong light and tested in strong light increased photosynthetic rate with continued exposure to light for a period of some ten hours. Those cultivated in weak light and tested in strong light attained their maximum within one hour, and declined rapidly thereafter. As the light intensity under which they were cultivated increased, the slower were the plants to attain maximum photosynthesis in strong light. Any single species could be made to behave either as a sun plant or as a shade plant, depending upon the conditions under which it was grown.

The slowness of land plants to take advantage of sudden great increases in light is due in part to the differences in structure of leaves developed in shade from those developed in full sunlight. The internal surface of sun leaves may be as high as 20 times their external surface, that of shade leaves nine times their external surface (167). Moreover, the internal surface of sun leaves of xeromorphic structure goes as high as 22 to 31 times external surface, mesomorphic sun leaves, 12 to 16, xeromorphic shade leaves eight to ten. Turrell (168) found that leaves of species that are xeromorphic in high light intensities with respect to the ratio of internal to external area, in low light intensity tend to be mesomorphic.

The tendency of many plants to show a decline in photosynthetic rate at high light intensity serves to minimize the differences in growth rate between shaded and unshaded plants. Stålfelt (160, 161), studying photosynthesis under natural conditions, found that on cloudy days photosynthesis in oats was positively correlated with temperature and light, but on hot bright days the stomata closed and photosynthesis followed the opening of the stomata rather than temperature and light.   In high light intensities he found a decline in the rate of photosynthesis in lichen caused by both high temperature and high light intensity. The temperature and light effects

were separable. Tests of photosynthetic rate in intermittent light have shown that when the periods of light and darkness are very short, several alterations per minute, photosynthesis increases markedly with respect to the total amount of light available. This behavior together with that reported by Harder (73) theoretically should enable plants receiving the highly variable light transmitted by the broadleafed forest canopy to grow more rapidly than the average light intensity would indicate. No demonstration has proved this to be true. Nägeli (110) considers sunflecks of negligible importance and diffuse sun and skylight to be chiefly responsible for growth beneath canopies.

The capacity of some plants to achieve a maximum rate of photosynthesis at lower intensities than others may be of some ecological significance. Dogwood, red oak and white oak were found to attain maximum photosynthesis at 3,000, 3,000 and 1,000 foot candles, respectively, whereas loblolly pine increased its photosynthetic rate up to 9,000 foot candles. Kramer and Decker (87) give this as one reason for the ability of hardwoods to invade pine stands, where the maximum light intensity averaged about 4,500 foot candles. Pines, on the other hand, do not invade oak, for there the maximum intensity was only 1,900 foot candles.

Vegetation beneath forest canopies tends to disappear at light intensities below 4% of full sunlight. The density required to exclude herbs beneath deciduous canopies is considerably lower than that required beneath evergreen canopies, probably due to the start such plants can get in the spring before the hardwood leaves are fully developed. The extent to which a forest species can reduce sunlight through the density of its canopy is an indication of its relative capacity to perform photosynthesis at low light intensity. This indication must be used with caution, however, as both conifer and hardwood leaves tend to remain attached on the lower branches even though they consume more photosynthate than they produce.

Just as in forests vegetation tends to disappear at light intensities below 4% of total sunlight, so also water plants tend to disappear or at least to lose their chlorophyll when exposed to intensities of less than 4% (121). Furthermore, when light intensities decline in lakes due to an increase in turbidity, the depth to which plants will grow also declines (120). Manning and Juday (92) found that the chlorophyll content was a fairly good index of the photosynthetic capacity of submerged water plants.

Loose, Pearsall and Willis (90) found that photosynthesis in *Chlorella* occurred down to a depth of eight meters in the Windermere where the light intensity was 5% of full sunlight. The actual compensation point at which photosynthesis balanced respiration was at 2% of full daylight; hence, theoretically the plants could grow at ten meters' depth. But other factors were unfavorable at this depth. No rooted plants grew below 4.3 meters. Extensive studies of photosynthesis in Lake Erie indicated that the compensation point occurred at 2% to 3% of full sunlight (100). This being so, plants should be able to survive down to depths of eight meters, but because of turbidity light intensity is frequently inadequate at these depths. The depths of Wisconsin lakes at which the maximum rate of photosynthesis occurred in *Chlorella* and *Coccomyxa* were found to be five to six meters in a transparent lake and .25 meter in a lake having highly colored water (84). The maximum percentage utilization of solar radiation occurred at ten meters' depth in the very transparent water and at one meter in the highly colored water.

INTERRELATIONSHIP BETWEEN LIGHT AND OTHER FACTORS

Temperature. As less than 1% of the energy which leaves absorb is used in the photosynthetic process, they obviously would soon become overheated were this absorbed heat not dissipated in some manner. The temperature of assimilating leaves under ordinary conditions remains very close to that of the surrounding atmosphere, generally being slightly below air temperature. Temperatures have been reported, however, as much as 7° C. below and 40° C. above air temperature (174). Three methods of heat loss operate to keep leaves very close to air temperature. These are radiation to surrounding objects and to space, convection loss to surrounding air, and heat loss through vaporizing water in transpiration. Curtis (43) believes transpiration to be of minor importance in maintaining leaf temperature, inasmuch as he was unable to demonstrate that high temperatures built up in thin objects that were free to lose heat through radiation and convection. Others have insisted that transpiration plays a dominant role in maintaining leaf temperature (174). It is generally conceded that transpiration may lower leaf temperature 2° to 3° C., or even more (150). As is well known, temperature exerts a major influence

on all biochemical processes in plants. For most there exist minimum, optimum and maximum temperatures. Ashby and Oxley (11) carried out an extensive experiment on the relationship of light intensity and temperature to the assimilation rate and multiplication of fronds in *Lemna*. Sixteen combinations of light intensity and temperature could be studied on 16 separate colonies simultaneously. Experiments were performed at 10°, 18°, 21°, 24° and 29° C., and at light intensities of 80, 150, 350, 500, 750, 900, 1,100 and 1,600 foot candles of light. This gave a total of 40 different combinations of light intensity and temperature. Each experiment lasted from 17 to 20 days. These extensive data enabled them to work out equations showing the relationship between frond multiplication and light intensity. Frond multiplication rate was almost directly proportional to light intensity except at the two lowest intensities. It tended to increase at any specific temperature with an increase of light intensity, and this was more pronounced at high temperatures than at low temperatures. Assimilation rate increased with temperature, from 10° to 29° C., provided only that the light intensity was adequate. The increase was insignificant at 80 and 150 foot candles. From these data they were able to construct three-dimensional models showing the combined effects of light and temperature on the rate of multiplication and net assimilation rate of the fronds.

Ahmad (1), working with *Azolla*, obtained comparable results. For a series of temperatures varying from 7° to 36° C., the growth of *Azolla* increased with increasing light intensity from 35 to 191 foot candles. For each light intensity, growth increased with temperature up to 20° C. and declined at higher temperatures. He presumed that the decrease in growth at higher temperatures was due to increasing respiration, rather than a decrease in photosynthesis. The point at which respiration overbalanced photosynthesis occurred at about 25° under illumination of 35 foot candles, at 35° for 72 foot candles, and was not attained at all for the two higher light intensities, though the values were approaching the compensation point at 36° C.

In contrast to the foregoing experiments in which a single measurement of growth or photosynthesis was made after a definite period of time, are those of Stålfelt (160, 161) involving a series of determinations at short time intervals on the rate of photosyn-

thesis at specific temperatures and light intensities. Both high temperature and high light intensity would produce in *Usnea dasypoga* a relatively high rate of photosynthesis following the initial exposure, but this gradually declined with continued exposure. The optimum temperature for photosynthesis therefore was dependent upon the duration of exposure, and gradually receded with lengthened exposures until the temperature reached 21° C., which proved to be a true optimum for prolonged exposure. Inasmuch as respiration is increased by temperature, the optimum value for apparent assimilation is the result of the action of the inhibiting effect of temperature on photosynthesis and its stimulating effect on respiration. Light acted similarly on photosynthesis. The stronger the light, the quicker the inhibiting effect becomes evident, and the longer light acts the more the rate of assimilation is decreased. Light has a marked influence in increasing the rate of respiration of both green and etiolated tissue (177).

Mineral nutrition. Light, through its effect on transpiration, influences the uptake of mineral salts, and, through its effect on photosynthesis, influences the use of these salts in general metabolism and in the formation of plant tissue. Sun leaves are greater than shade leaves in both fresh and dry weight and in ash (24). Calcium tends to accumulate in proportion to amount of transpiration but potassium does not. Light also increases ash accumulation of plants independent of transpiration, potassium in this instance being affected more than calcium. McCool (98) reports that the injurious effects of manganese on soy beans, snap beans and tobacco could be mitigated by shading, an indication that less manganese was taken up in the shade than in full sunlight. Broyer and Hoagland (25) noted that roots low in salt content and high in sugar have a high potential for salt absorption and in such condition absorb salt at approximately the same rate, irrespective of light intensity and transpiration. But when they have a high salt and low sugar content, salt absorption is more closely related to transpiration. They concluded that transpiration affects but does not govern salt movement.

Low light intensity causes starvation resulting from inadequate supply of the products of photosynthesis. This induces other nutritional disturbances including unbalanced utilization of essential mineral elements. Fred, Wilson and Wyss (59) reported

that soy beans growing in a soil poor in nitrogen but inoculated with root nodule-forming bacteria appeared to suffer from nitrogen hunger if the light intensity was high. This condition could be relieved either by lowering the light intensity or by applying nitrogen to the soil. Best results were obtained by keeping the light intensity constant and applying nitrogen to the soil, but by shading the plants for twelve days and then returning them to the sun good growth and nitrogen fixation occurred. Once a plant got a good start and adequate root nodules were formed, they were able to grow at a satisfactory rate in full sunlight. Gast's (63) very extensive study of the effect of nitrogen and light intensity on the growth of pine seedlings enabled him to develop three-dimensional models showing the relationship between these respective growth factors. If plants are starved for nitrogen they are unable to make adequate use of high light intensity, and, on the other hand, if they are cultivated in low light intensities they are unable to respond by increased growth to a more generous nitrogen supply. Where nitrogen supply was adequate, growth continued to increase up to the full intensity of sunlight. Mitchell (103) found that the total nitrogen content of seedlings cultivated under different degrees of shade tended to increase almost directly with increasing dry weight. Phosphorus content also increased with dry weight, but not to the same extent, whereas total potassium content tended to remain constant, irrespective of light intensity. Or, expressed as percentages of dry weight, potassium and phosphorus decreased with increasing light intensity, but nitrogen tended to remain more or less constant.

Extensive studies of the interaction of factors in the growth of *Lemna* reveal that the optimum nitrate concentration in the growth medium increases with increasing light intensity (180, 181, 182). At the highest light intensities, a decrease in nitrogen supply resulted in an increase in frond numbers but low net assimilation rate, smaller frond area, less protein content and loss of chlorophyll. At the lowest light intensities a decreasing nitrogen supply also increased frond numbers but in marked contrast to the behavior at high intensity, caused an increase in net assimilation, larger frond area, greater protein content, and deeper frond color. This, of course, was simply a reaction to oversupply of nitrogen at the lower light intensities. Both of these factors influenced the basic metab-

olism of *Lemna*. Plants starved for nitrogen had a lower respiration rate than those adequately supplied with nitrogen. The respiration rate also decreased regularly with decreasing light intensity from 1,200 to 900, 600 and 300 foot candles. The decrease in respiration of plants grown in low light intensities is presumed to be due to a decrease in carbohydrate level. In other words, it is a starvation effect. In nitrogen-starved fronds, respiration is affected by two opposing tendencies, that of low nitrogen supply to depress respiration, and that of high carbohydrate concentration to accelerate respiration. The curve of respiration of colonies with full nutrient supply under different light intensities was the same as that for nitrogen-starved colonies, and both were similar to that for real assimilation. Similar disturbances in metabolism and nutrient balance occur in other plants.

Borden (23) found that sugar yields from Hawaiian cane were adversely affected if sunlight conditions were such as to limit the complete assimilation of the applied nitrogen and potassium fertilizers. Nitrogen seemed to be mainly responsible for the decreased sugar yield, but at the same time there must be a proper balance between nitrogen and potassium to obtain cane with the best quality juice. Both nitrogen and sunlight must be adequate for good growth (22).

Blackman and Templeman (21) report that leaf production by *Agrostis tenuis* and *Festuca rubra* subjected to frequent defoliation was markedly increased by applications of ammonium sulphate or calcium nitrate, provided the plants were exposed to full daylight, but if they were shaded these same fertilizing treatments depressed leaf production. Light intensity in this instance greatly altered the ratio of organic acid to carbohydrates. This study confirmed earlier work by the same authors (20) that generous applications of ammonium sulphate and calcium nitrate depressed leaf production of frequently defoliated grasses in 37% and 63% daylight. The lower light intensity interfered with the elaboration of protein, and nitrate tended to accumulate in the plant tissue. The gains in protein as a result of fertilizing in full daylight resulted in a decrease in percentage of carbohydrate content, but at the lowest light intensities little effect on carbohydrate level was brought about by fertilizing. These writers ascribe the effects of light intensity and nitrogen supply on leaf metabolism and leaf production in part to

their indirect effects on root metabolism. The shaded plants, as is well known, tend to use more of their organic food substances to produce leaf and stem tissue than root tissue.

Quite a different effect is that reported by Heinicke (77) on prolonging leaf functioning through autumn application of nitrogenous fertilizer to apple trees, thereby preventing yellowing and early abscission of leaves. With such treatment the foliage remained dark green and stayed on the twigs until temperatures dropped well below freezing. Photosynthesis in excess of respiration continued up to early November. In contrast, the yellowish leaves of the unfertilized trees showed a marked decline in rate of photosynthesis which after October 22 fell below the rate required to balance respiration. Twigs from trees that were fertilized in late fall also proved more winter hardy than those from the unfertilized trees.

Water relations. It has long been recognized that transpiration is affected very strongly by light intensity. Martin (93), in a careful study with sunflower plants exposed to 6%, 19%, 40%, 67%, 70% and 100% solar radiation at noon, found transpiration to be a linear function of radiation intensity. The slope of the line was inversely proportional to the age. The intercept on the transpiration axis and the fraction of transpiration, 38% to 81%, due to the direct effect of radiation, both depended upon the evaporating power of the air.

A very excellent study of the combined effects of varying light intensities and soil moistures was made by Clements and Long (37). Sunflower plants were grown under shades transmitting 11%, 19%, 27%, 44%, 65% and 100% light intensities, and in soil moistures of 14%, 18%, 26% and 35%. At each soil moisture, growth in terms of dry weight increased with increasing light intensity up to full sunlight, with the single exception of 14% moisture. And for each light intensity the growth increased with soil moisture up to the highest value used. The water requirement, that is, the water evaporated per gram of dry weight, was greatest in 11% light and 35% soil moisture. It was least at 100% light and 14% soil moisture. This very significant study has important implications in considering the relative importance of various factors in the growth of plants under natural conditions. Plants grown in high light intensities have long been known to have

higher percentage dry matter than those in low intensity (89). Heinicke and Childers (78), studying apple leaves, found that the rate of photosynthesis gradually declined as the moisture content of the soil decreased. They also found, however, that the early closing of stomata associated with reduced water supply tended to conserve water to a greater extent than it reduced photosynthesis.

Extensive studies by Schneider and Childers (142) show that photosynthesis of apple leaves is very closely correlated with transpiration. As the soil gradually dried out there was, first, a reduction in rate of transpiration, and one day or more later, a reduction in photosynthesis. A marked reduction in both photosynthesis and transpiration and an increase in respiration took place before wilting was evident. Fairly high rates of photosynthesis were obtained on several occasions when the stomata appeared to be closed.

Growth, form, and substance. Growth is the net result of the combined action of all growth factors. As the preceding discussion has brought out, maximum growth is dependent upon a favorable combination of all. When all other factors are present at appropriate levels, many temperate zone plants, particularly woody plants with thick crowns, are able to increase their growth rate with increasing light up to the full intensity of natural sunlight (68, 99, 106, 152). With such plants some of the leaves at any one time may be receiving more light than they require and others less; also, within individual leaves, some of the chloroplasts may be receiving more light than they require and others less. It is the combined functioning of all the chloroplasts in all leaves throughout the day and growing period that determines total photosynthesis and, in consequence, the growth rate.

Thut and Loomis (163) studied the effect of light and temperature on stem elongation of asparagus, field bindweed, castor bean, and on leaf elongation of maize. When soil moisture was adequate and relative humidity high, growth followed the temperature curve very closely. When moisture supply was inadequate either because of low humidity or low soil moisture, growth was checked during midday. These workers conclude that the four plants tested are never inhibited in growth rate by the action of light directly. Indirectly, light increases growth by increasing the rate of photosynthesis and by increasing temperature.

Benedict (18) cultivated three species of range grass in 28%, 45%, 57% and 100% light from June 7 to October 1, at Cheyenne, Wyoming, where, due to high elevation and infrequence of cloudy weather, the total light received is high. All degrees of shading caused increases in height but decreases in dry weight. McCalla and his co-workers (97) attempted to determine by multiple correlation analysis the relationship of growth to light and temperature for maize and gladiolus. They report that growth was directly correlated with temperature and inversely correlated with light. No measurements of light intensity were taken. Growth was measured at four-hour intervals throughout the course of the day and night. They noted a tendency of light to depress growth during midday, very likely due to a water deficit. The actual growth, however, frequently was just as great during midday as at any other time because then the temperature was most favorable.

Gast (63), in careful work with pine seedlings in nutrient cultures, found that the dry weight produced during a growing season could be expressed by the equation,

$Y = A \log R + B$ where
Y = dry weight in grams
A = a constant determined by species
R = cumulative solar radiation in gram calories per square centimeter
B = a constant dependent on species and initial seed weight

The validity of this relationship was further confirmed by Mitchell and Rosendahl (106).

Because few plant habitats present optimum conditions of growth factors other than light intensity, some degree of shade is not always harmful and frequently proves beneficial. Neel (112) found that the yield of a pasture in terms of days animals could be grazed and the total gain in weight of the animals was not reduced significantly by the shade of locust or walnut trees up to an age of 13 years. The shade, however, was kept moderate by pruning the trees. The percentage of blue grass tended to increase as the shade developed. Two important tropical crops, coffee and cacao, do not thrive well without shade. Normally, this is provided by growing them beneath the shade of such light-foliaged trees as *Inga* and *Erythrina*. Arrilaga and Gomez (7) found that Arabian coffee grown under lath shade in Puerto Rico did best with one-third sunlight, but that yield was approximately equal with one-third and one-half sunlight. With two-thirds light, the trees were sickly and the

yield low. Cobley (38) cultivated cacao in one-fourth, one-half, two-thirds and full sunlight in Trinidad. Optimum growth occurred in one-fourth sunlight. This was many times greater than growth in full sunlight.

Very intense light, especially that containing a strong component of ultraviolet, tends to destroy chlorophyll and may lead to the death of plants. Light tends to stimulate respiration both of green and of colorless tissue (57, 65, 130a). The ability of different species of plants to develop protective devices against too intense light varies greatly, and to this variation Collaer (39) has ascribed the ability of *Pinus cembra* to survive at higher elevations than *Picea excelsa*. Such devices include all sorts of mechanical means of protection against water loss and against excessive absorption of radiation, such as waxy bloom, hairiness, thickness of leaves, orientation of leaves towards the light, and light-diffusing tissue (123, 113a). They also include changes in pigment concentration, chloroplast arrangement and the presence of protective pigments (61). The water plant *Potamageton,* for instance, has both floating and submerged leaves. The floating leaves have a strong red color due to the presence of carotin granules, whereas the submerged leaves of the same plants are green in color. Arens (6) has found that if the red floating leaves are submersed or cultivated in reduced light intensity, they turn green, whereas the green submersed leaves, if placed on the surface or cultivated in high light intensity, turn red.

Langham (88) has found that prostrate forms of several grasses, together with corn and rice, are negatively phototropic under high light intensities and positively phototropic when shaded by burlap. All tended to grow erect in darkness. Leaves show remarkable increases in thickness, intercellular space, size of cells and stomata, and layers of assimilating tissue in high light intensities (82).

Of particular importance is the effect of light on root development and shoot-root ratios. Blackman and Templeman (21) present evidence that nitrogen supply and light intensity markedly affect the leaf-root ratio, and that at low light intensity leaf production takes place at the expense of roots. The rooting of slash pine was found by Mitchell (104) to be favored by high light intensity, high humidity, and temperatures of 65° to 75° C. The light intensity in this case undoubtedly acted through its influence on the

available photosynthetic food supply. Length of *Lemna* roots was found by White (181) to be directly related to light intensity over a range of 50 to 300 foot candles, irrespective of the level of potassium supply. Reduction in either light or potassium supply at all levels, however, did tend to decrease root length, but with potassium this appeared to be an indirect effect caused by a decrease in the net assimilation rate.

Light has other important effects on the development of plants. Those kept in darkness remain colorless, fail to unfold leaves and have excessively elongated stems. Both blue and red light are effective in preventing etiolation, their effectiveness increasing with intensity (56). Infrared, on the other hand, stimulates elongation. Platt (125) has found that the tendency of certain wheat varieties to develop solid stems is favored by high light intensity. Apaoan (5) reports that shading reduced the growth of tillers and delayed the development of rice plants. It also resulted in poor seed. Plants fail to flower under temperature and day length conditions normally adequate for flowering, if the light intensity happens to be too low. *Chrysanthemum* and *Euphorbia,* for instance, failed to flower when the maximum intensity of light to which they were exposed had been reduced to 500 foot candles by cheesecloth shade (129). Light appears to influence the proportion of cleistogamous flowers produced by various plants. Trent (166) reports that in 50% light intensity *Specularia perfoliata* produced cleistogamous flowers but no open flowers; in full sunlight it produced both types.

Chlorophyll, the carotinoids, the anthocyanins and various other pigments and substances that play an active or protective role in the life of the plants are profoundly influenced by light (3, 115). Of great importance to animals is the influence of light on the formation and accumulation of various vitamins in plant tissue, particularly vitamin D and ascorbic acid (70, 132, 133, 134, 135, 137). Shaded potato plants produced tubers low in specific gravity, dry weight and mealiness, and if grown with a high nitrogen supply, incidence of blackening in the tubers was increased (111). Arrilaga and Villamie (8) report that oil in lemon grass is produced in the highest amount and highest quality by the full intensity of tropical sunlight.

Plants inadequately nourished because of low light intensity tend to be more susceptible to noxious gases such as SO_2 (145) and to

infection by plant diseases (75, 76). Cotton bolls tend to shed if kept for as long as three days in darkness or under illumination of 50 foot candles (48). Shading was found to be particularly harmful after flowering.

LIGHT AND SUCCESSION

Light intensity is only one of several factors influencing plant succession. It probably becomes dominant only after succession has advanced to such a stage that the plant cover is sufficient to reduce light intensity to below 20% of full sunlight. Inadequate nutrient and moisture supply and inadequate protection against moisture loss, extremes of temperature, unstable soil and wind action may prevent plants from colonizing open areas. Yet when these same areas are given some protection by shading, vegetation may become established and thrive (117). Such stages of succession are clearly governed by factors other than light intensity. Certain herbs and field crops, notably red clover, are capable of growing so rankly that they shade out white clover or other low-growing plants, but ordinarily it is not until the advent of woody plants, both erect and climbing forms, that shade begins to play an important role in succession. Pioneer woody species such as gray birch, eastern red cedar, the Rocky Mountain junipers and pinon pine rarely cast a dense enough shade to preclude invasion by other species. Their shade and root competition may, however, markedly reduce the growth of invaders, but as the pioneers drop out seedlings of longer-lived species take over. So succession proceeds from the thin-foliaged scattered stands to the thick-foliaged dense stands. And as it proceeds, competition for light becomes more and more keen. Under these conditions the plants that actually attain a foothold beneath forest canopies are those species whose seeds are able to germinate and whose seedlings are able to persist for long periods in light of low intensity. Seedlings of such species —usually already established on the forest floor—are the ones that as a rule pre-empt the space left by the death of veterans and ultimately form the climax. But when timber cutting, windstorm, insects or disease open up large areas of forest, these advance seedlings, suddenly exposed to full sunlight, may succumb because of unbalanced root development and tender leaves.

Ståfelt (159) has shown that branches and twigs of Norway

spruce that had been subjected to shade for a considerable period of time reacted in three ways to thinning. Branches that had been heavily shaded died when exposed to intense light; those formerly receiving abundant light opened normal buds and developed adventitious buds which, without thinning, would have remained dormant. On the other hand, branches which had been growing in weaker but still favorable light before thinning continued growth, but with development of heavier foliage. The old shade needles frequently dropped off completely and the new ones developed after the thinning showed an increase in diffusion capacity.

Closely related to natural succession are the successions artificially induced when foresters plant trees on lands occupied by other plant associations. Filzer (54) studied the behavior of the Scotch broom association in the Swabian Alps as modified by planted pines. The pines did not cause complete elimination from the area of plants characteristic of the Scotch broom association. Only those unable to endure the shade of the pines died. The same sort of selective succession occurred also where pines were planted on land formerly dominated by heather. The associations brought about by planting, therefore, were heterogeneous containing, in addition to the planted trees, those elements of the original association that could withstand the shade, plus new species capable of invading the pine community but unable to invade the original association.

The controversy over which is the more important, light or root competition, in determining seedling establishment and the course of natural succession beneath forest canopies is still a live one. But work during the past ten years goes far to clear up misunderstandings. The controversy has pivoted around whether the concept of tolerance as used in forest and ecologic literature should be defined as the capacity to endure shade, or the capacity to endure all factors of competition including root competition for moisture and nutrients (85, 164). In recent discussions of this question, Shirley (151, 152) points out that the relative capacity of jack pine, red pine, white pine and white spruce to endure shade in the Lake States increases in the order named and that natural succession proceeds in this order. Their relative juvenile growth rates and vigor, irrespective of light intensity and resistance to drought, in soils deficient in nitrogen and phosphorus, are in the

exact opposite order. Resistance to heat injuries and recovery therefrom, and establishment, survival and root penetration of first year seedlings are species characteristics which are independent of light, moisture and nutrient requirements.

Harley (74) reports that European beeches grown under artificial shades transmitting 10% light resembled those grown in natural woodland shade in having relatively high water content, poor root growth, low dry weight of both root and shoot, low ratios of root to shoot, incomplete mobilization of nitrogen before the fall of the cotyledons, and a very low rate of nitrogen absorption. Harley concluded: "These experiments therefore indicate definitely the immense importance of light intensity under woodland conditions, and also show that the complications arising out of soil composition and structure are of secondary importance at the early stage of the life of the beech".

Shirley (152) reports on extensive experiments in which both light intensity and root competition were varied. At each light intensity growth could be increased by decreasing root competition, and at each level of root competition growth could be increased by increasing light. However, the increase in growth rate never was as great where only one factor was improved as where both were improved.

Low light intensity, through stimulating top growth at the expense of root growth, tends to increase susceptibility to summer drought in regions of deficient rainfall (19, 69). The preponderance of evidence seems to be that the requirements of the several species for moisture, nitrogen, light and other growth factors need not be confounded (66, 105, 117, 118, 122). Precision demands that their effects be considered individually and in relation to one another and not as a single, complex, inexplicable entity.

There is an effect of root competition quite aside from that due to competition for moisture and nutrient supply that merits consideration. This is the so-called "root antagonism"—the inability of one plant to thrive when its roots grow in soil permeated by the roots of certain other plants. Interest in this subject, long dormant after it was first discovered, has been revived by agronomists because of its importance in pasture grass management. Schreiner (143, 144) has found that sod inhibits growth of hybrid poplars planted in fertile, well-watered soils. He has also discovered that

the effect could be produced by growing poplars in nutrient solutions that first had passed through cultures in which grasses were growing, even though the solutions were renewed before showing detectable depletion of nutrient elements. Coster (41) reports that a rich and varied undergrowth occurred beneath a dense canopy of *Lagerstroemia stegoisa* and *Ficus kurgii*, whereas no undergrowth occurred beneath a canopy of *Swietenia macrophylla* transmitting two to three times as much light. Coster attributes the difference to shortage of water caused by the strong root competition of mahogany, but root antagonism also may have been a factor.

This further emphasizes the desirability of seeking through factorial design of experiments and multiple correlation analysis, to work out the true interrelationship of the several growth factors of plant habitats. To confound them with one another inevitably confuses thinking, thereby adding mystery to subjects open to clear analysis. Succession can best be understood by accurately appraising the several growth factors of the habitat and understanding the respective requirements of the several species involved for light, moisture, heat, nutrients and other survival and growth elements.

As, more and more, man seeks to use the vegetation of deserts, fields, grasslands and forests for economic ends, the more important it becomes that he understand the action and interaction of the several growth factors in determining the composition, growth, vigor and perpetuation of plant communities. Rapid strides have characterized research of the past ten years. The coming decade should see this and new work integrated into handbooks and guides for the successful management of natural vegetation. Only then does the information become available to the practitioner and thereby serve its fullest usefulness. And only then can the research worker quickly appraise the status of information on a specific type or association and thereby direct his research work to those subjects most in need of further study. Precise ecologic information grows in importance and usefulness yearly. Its diligent search and collation is a worthy scientific and public service.

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THE BOTANICAL REVIEW

CYTOTAXONOMY OF NICOTIANA

T. H. GOODSPEED
University of California, Berkeley

CONTENTS

INTRODUCTION

During the past fifteen years there has accumulated a body of new morphological, distributional and cytological information concerning the some 60 valid species of the genus *Nicotiana* sufficiently large and sufficiently significant to become subject to review along with cytotaxonomic conclusions which have been derived from it. In the University of California Botanical Garden studies of the genus *Nicotiana* were first undertaken by the late Prof. W. A. Setchell over 35 years ago (134) and have been carried on continuously since that time. Originally they had to do with genetic analysis of numerous varieties of *Nicotiana tabacum* (82, 136) and

with the character of *Nicotiana* species used or cultivated in the
pre- and post-Columbian periods (135). Later the scope of the
Nicotiana investigations was broadened to include cytogenetic and
other studies of the origins and relationships of all the species of
the genus. To this latter end an almost complete living collection
of species and varieties of *Nicotiana* has been grown for many years.
In addition to the *Nicotiana* investigations just mentioned the only
other comprehensive ones of like nature are those which have been
carried on in Russia and Bulgaria by Dr. D. Kostoff and his
assistants.

As will be indicated below, the majority of *Nicotiana* species
are today found in temperate South America. In order to de-
termine their distribution, the extent of their natural variation and
the possible occurrence of previously unknown species, three expe-
ditions have been sent to South America from the University of
California Botanical Garden during the past ten years. Members
of these expeditions made numerous collections of herbarium speci-
mens and seeds of species of *Nicotiana* in Peru, Bolivia, Chile,
Argentina and Uruguay. Additional material has been received
from collaborators in South America and in Australia and the
South Pacific. These accessions have included a number of previ-
ously unknown or little known species grown and investigated for
the first time in the University of California Botanical Garden;
among them are *N. benavidesii* (53, 56), *N. cordifolia* (48, 54, 56),
N. knightiana (53, 56), *N. thyrsiflora* (53, 56), *N. setchellii* (55,
56), *N. otophora* (56, 66), *N. wigandioides* (48, 49, 56), *N.
arentsii* (57), *N. corymbosa* (48, 54, 56), *N. spegazzinii* (56),
N. linearis (54, 56), *N. suaveolens* (48, 68, 153, 154), *N.
exigua* (153, 154), *N. maritima* (50, 152, 153, 154), *N. velu-
tina* (152, 153, 154), *N. gossei* (154), *N. excelsior* (153, 154),
N. megalosiphon (48, 152, 153, 154), *N. goodspeedii* (50, 152, 153,
154), *N. occidentalis* (154), *N. rotundifolia* (48, 50, 152, 153, 154),
N. debneyi (48, 50, 152, 153, 154) and *N. fragrans* (154).

Following brief summaries of the taxonomy and cytology of the
genus *Nicotiana,* the cytotaxonomy of the three subgenera will be
reviewed and commented upon. Where no citation of literature
accompanies factual information or conclusions drawn therefrom
reference is made to material not as yet published by the author
and his associates. To avoid repetition of citations and otherwise

to conserve space, references to the literature concerning the cytology of the numerous interspecific hybrids of *Nicotiana* are largely confined to the list of such hybrids on p. 579.

TAXONOMY

The genus *Nicotiana* has been referred to Tribe Cestrineae by Bentham and Hooker and to Tribe Cestreae, Subtribe Nicotianinae, by Wettstein. Unlike some other members of the Solanaceae, no specialized feature distinguishes it from other genera. In the Bentham and Hooker (7) classification *Nicotiana* stands closest to *Fabiana, Vestia* and "*Dittostigma*" (probably *Nicotiana longiflora* var. *acutiflora,* according to Millán 113); in Wettstein's (151), *Petunia, Bouchetia, Nierembergia* and *Parabouchetia* are added to these. Morphological resemblance between Solanaceae and Scrophulariaceae, an acknowledged feature of the tribes Cestreae and Salpiglossideae (126), is not consistent nor pronounced in *Nicotiana,* although it occurs.

Taxonomically the Linnean genus *Nicotiana* today remains intact, although the many species added in the interim have considerably broadened its definition. Between 1781 and 1874, 13 attempts were made to erect genera for particular *Nicotiana* species, and one of these, *Lehmannia* of Sprengel (142), survived as late as 1899 (28). The suggestion that the genus *Petunia* should be included under *Nicotiana* (151) has not been accepted, although Fries (42) recognized in *Petunia* the subgenus *Pseudonicotiana* comprising the most *Nicotiana*-like *Petunia* species.

The treatment of *Nicotiana* by Bentham and Hooker, while not entirely complete for inflorescence, is a useful short diagnosis of the genus. Rather comprehensive descriptions of the classical species are given by Don (30), Dunal (32), Comes (28) and Grabovetzkaya (72). The first taxonomic organization to embrace many species of the genus was that of Don (30), in which four sections—*Tabacum, Rustica, Petunioides* and *Polydiclia*— were recognized. Wettstein's (151) reduction to three sections by elimination of *Polidiclia* has been generally accepted. Only Gray (74) has abandoned emphasis on corolla shape and color in favor of capsule dehiscence.

On purely morphological grounds Splendore (141) in 1906 and Anastasia (1) in 1914 postulated the hybrid derivation of certain

species of *Nicotiana* and advanced hypotheses concerning the phylo-genetic structure of the genus. Between 1912 and 1930 a number of investigators (*cf.* 16, 34, 70, 71, 83) supplied information on the cytology and genetics of many species and thereby implemented a mode of attack upon phylogenetic problems in *Nicotiana* which Splendore and Anastasia had anticipated. East (34) referred to "a number of genetic centers which may possibly be made the basis for various genetic subdivisions when our information is more complete". Goodspeed (50) crystallized and expanded this "gene-tic group" concept, applied it to the entire genus and contrasted clusters of related species with amphidiploids.

Recently Kostoff (97, 98) has set aside the Donian organiza-tion of the genus in favor of eight "sections", to seven of which he attaches the designations given by Goodspeed (48, 50, 56) to the "genetic groups" that the latter described, although the com-position of certain of Kostoff's "sections" is not the same as that of the "genetic groups" carrying the same designation. By con-trast, the following classification proposed by the author (58) retains, as did Wettstein's three of Don's major subdivisions, refer-ring to them as subgenera and recognizing within each of them a series of sections, some of which, in general, correspond to the author's "genetic groups", others to amphidiploid species, while still others are of a more inclusive nature. This classification re-flects to some extent the cytological evidence reviewed in this article.

Of a considerably greater number of species of *Nicotiana* that have been described, only the following 58 are here recognized as valid. As will be noted below, chromosome number and mor-phology are known in 55 of these species, and 53 of them have been involved as parents in F_1 interspecific hybrids, the meiotic chromosome behavior of which has been analyzed (*cf.* p. 579). Three of the species listed, *N. ameghinoi* Speg., *N. ingulba* Black and *N. stenocarpa* Wheeler, are presumably known only from the wild state, and their cytology has not been investigated. The validity of *N. frigida* Phil. (54), closely allied to members of the Acuminatae, is not entirely clear, and this species is not therefore being retained here.

Subgenus *Rustica*

Section Paniculatae: *N. paniculata* L., *N. knightiana* Goodsp., *N.*

solanifolia Walp., *N. benavidesii* Goodsp., *N. raimondii* Macbr., *N. cordifolia* Phil., *N. glauca* Grah.
Section Thyrsiflorae: *N. thyrsiflora* Goodsp.
Section Rusticae: *N. rustica* L.

SUBGENUS *Tabacum*

Section Tomentosae: *N. tomentosa* R. & P., *N. tomentosiformis* Goodsp., *N. otophora* Griseb., *N. setchellii* Goodsp., *N. glutinosa* L.
Section Genuinae: *N. tabacum* L.

SUBGENUS *Petunioides*

Section Undulatae: *N. undulata* R. & P., *N. wigandioides* Koch & Fint., *N. arentsii* Goodsp.
Section Trigonophyllae: *N. trigonophylla* Dun., *N. palmeri* Gray.
Section Alatae: *N. alata* Link & Otto, *N. langsdorffii* Weinm. apud Roem. & Schult., *N. bonariensis* Lehm., *N. longiflora* Cav., *N. plumbaginifolia* Viv., *N. sylvestris* Speg. & Comes, *N. repanda* Willd., *N. stocktonii* Brandeg., *N. nesophila* Johnst.
Section Noctiflorae: *N. noctiflora* Hook. (syn. *N. cavanillesii* Dun.), *N. petunioides* (Griseb.) Millán (syn. *N. pampasana* O. Kuntze), *N. ameghinoi* Speg., *N. acaulis* Speg.
Section Acuminatae: *N. attenuata* Torr., *N. acuminata* (Grah.) Hook., *N. pauciflora* Remy (syn. *N. caudigera* Phil.), *N. corymbosa* Remy, *N. miersii* Remy, *N. linearis* Phil., *N. spegazzinii* Millán, *N. clevelandii* Gray, *N. bigelovii* (Torr.) Wats., *N. nudicaulis* Wats.
Section Suaveolentes: *N. suaveolens* Lehm., *N. maritima* Wheeler, *N. velutina* Wheeler, *N. exigua* Wheeler, *N. gossei* Domin, *N. excelsior* Black, *N. benthamiana* Domin, *N. megalosiphon* Heurck & Müll. Arg., *N. goodspeedii* Wheeler, *N. occidentalis* Wheeler, *N. rotundifolia* Lindl., *N. debneyi* Domin, *N. fragrans* Hook., *N. ingulba* Black, *N. stenocarpa* Wheeler.

Of the species listed above, three have previously been dealt with cytologically and otherwise under different designations by a number of investigators. Priority of publication necessitates the replacement of *N. pampasana* O. Kuntze by *N. petunioides* (Griseb.) Millán, and *N. caudigera* Phil. by *N. pauciflora* Remy. In addition, it appears necessary with the evidence at present available to replace *N. cavanillesii* Dun. by *N. noctiflora* Hook.(*cf.* 73).

CYTOLOGY

Taxonomy has some reflection in the chromosome number constitution of the genus *Nicotiana*. For the Solanaceae (exclusive of *Nicotiana*), 12 pairs of chromosomes or multiples of 12 represent the mode, while 7, ca. 8, 9, 10, 11, 14, 17, 22, 30 and 34 pairs have also been reported (*cf*. 146). The chromosome numbers of *Nicotiana* species (18, 45, 48, 50) show a mode at 12 pairs with 9, 10, 16, (32 *cf*. p. 570), 18, 19, (154), 20, 21 (154), 22 and 24 pairs also occurring. This sequence of chromosome numbers is wide in range for a genus of the Solanaceae and includes certain numbers characteristic of Scrophulariaceae. Of the 55 species of *Nicotiana* for which chromosome numbers have been reported, 28 are 12-paired, eleven are 24-paired, four are 16-paired, three are 9-paired, two are 10-, 19- or 20-paired and the remaining numbers correspond to a single species in each case. Presumably all 24-paired American species are of amphidiploid origin. It has been suggested (16, 29, 87, 98) that 6 represents the basic chromosome number for the genus.

Chromosome morphology in most of the 55 species above referred to has recently been determined from comparative studies of pollen grain and root tip mitoses (50, 59, 154). In the case of a few species pollen grain mitoses alone have been investigated. The chromosomes of the genus are relatively small to medium in size (96). Although size distinctions within a genom or between members of different genoms are appreciable, the size range is not great. An average of 2.2 μ for the genom of shortest and 5.5 μ for the genom of largest chromosomes was previously reported (50), but it is now known (59) that the average chromosome size in *N. acaulis* somewhat exceeds 5.5 μ (*cf*. p. 567).

In *Nicotiana,* chromosome morphology, apart from differences in size, is sufficiently distinctive in many species to possess significance for interpretation of their origins and relationships. The chromosomes have been referred to (59) as median, submedian or subterminal where the arm ratio (length of long arm vs. that of short arm) is respectively 1:1, greater than 1:1 but less than 3:1, and 3:1 or greater. Simplifying the situation as much as possible for the moment by combining median and submedian positions of the centromere, it appears that for the genus as a whole these chromosomes predominate over subterminal ones in the ratio of approxi-

mately 5 to 3 (59). However, for each subgenus a distinctive ratio obtains. Thus, in subgenus Rustica the ratio is 9 to 1; in subgenus Tabacum, 5 to 2; and in subgenus Petunioides, 4 to 3. Employing the term "karyotype" in accordance with Lewitsky's definition (106, cf. 4) to designate the physical properties of the chromosomes of a complement visible at somatic metaphase, it is impossible, therefore, to establish a common karyotype pattern for Nicotiana (59). The suggestion (107, 131) that the possession by a species of Nicotiana of a genom consisting largely of median chromosomes connotes a more primitive rather than a derived condition, is largely borne out by other evidence.

It is to be noted that in Nicotiana the region of spindle attachment is localized in a chromosomal organ, the centromere. The genoms of all species show at least one pair of chromosomes bearing proximal satellites. The satellite thread may be long or short, and the segment may vary in size from just within the limits of visibility to approximately one-fifth the total length of the chromosome, distinctions in length of thread and size of segment being relatively constant specific characters. Frequently there are two satellited pairs (rarely one of these pairs with distal rather than proximal satellites) and, in the species of higher chromosome number, sometimes three satellited pairs. When only one pair is involved the satellites are characteristic of subterminal chromosomes, or, if no such chromosomes occur, of the short arms of a submedian pair. In species where two or more pairs are satellited the satellites involve a subterminal pair if present (cf. p. 571), and usually one or more submedian pairs, although in some instances both satellited pairs are subterminal. Strictly median chromosomes are rarely satellited.

Evidence of the relation of the nucleolus to the chromosomes in Nicotiana has been observed in only a few species. Kater (81) in N. longiflora traced the origin of the nucleolus to portions of telophase chromosomes. Correlation of the number of satellited chromosomes and the number of nucleoli at comparable stages (before fusion) and the presence of pycnotic regions associated with the nucleoli in resting nuclei have been observed in several species of section Alatae (3, 64)—N. alata, N. langsdorffii, N. longiflora, N. plumbaginifolia and N. sylvestris—and in the last named species the nucleoli have been seen at telophase to have their origin

in matrical material surrounding definite nucleolus-organizing regions of three chromosomes, two satellited and a third with pycnotic terminal region but no satellite. Gates (43) refers to the situation in *N. sylvestris* and interprets all three chromosomes as nucleolus-organizing. Additional constrictions for which a nucleolus-organizing function has not yet been demonstrated in *Nicotiana* occur in certain chromosomes (59, 154). Their presence is reflected in flexures distinct from flexures referable to coiling.

Studies of chiasmatype in species of *Nicotiana* are somewhat difficult because of the relatively small size of the majority of the chromosomes involved and their extreme contraction at the first meiotic metaphase (MI). Nevertheless, detailed knowledge of its karyotype permits both analysis of some to many of the bivalents of a given species and determination of the relation between somatic chromosome size or morphology, or both, and chromosome behavior at MI (50). Thus, a pair of either large or small median or nearly median chromosomes forms one chiasma in each arm to produce a ring bivalent. In such case, where small chromosomes are involved, terminalization is usually complete by MI, while it may not be entirely complete in bivalents of large chromosomes. In the case of submedian chromosomes a chiasma often appears only in the long arms and often is unterminalized at MI to give a characteristic cross-shaped bivalent. However, long chromosomes of this type frequently form two chiasmata, at times one in each arm. In the latter instance the chiasma in the short arms is usually terminalized at MI. Pairs of subterminal chromosomes, if short, commonly show a single, terminalized chiasma, but, if unusually long, may form two or, rarely, three chiasmata, in the last case including one in the exceedingly short arm or "head" (107).

The relation between these various chiasmatypes and distinctions in chromosome morphology is shown by comparison of chiasma frequencies with the ratios of median and submedian to subterminal chromosomes present. Thus, studies of four species of subgenus Rustica, five species of subgenus Tabacum and ten species of subgenus Petunioides show that the chiasma frequency per pair in the three subgenera is 1.87, 1.57 and 1.40 respectively (59). In other words, in the first subgenus where the genoms consist of chromosomes almost all of which are median or submedian, 87% of the bivalents show two chiasmata; in the second subgenus, where

the ratio of median or submedian to subterminal is 5 to 2, two chiasmata appear on 57% of the bivalents; and in the third subgenus, where approximately equal numbers of median or submedian and subterminal chromosomes occur, 40% of the bivalents have two chiasmata. The consistent relation between karyotype pattern, chiasma frequency and terminalization coefficient of the series of representative species studied has made it possible to predict with accuracy the character of pairing at MI in all other species for which the karyotype pattern is known.

Meiotic chromosome behavior of 131 F_1 interspecific hybrids of *Nicotiana* has been determined by the author and his associates. Other investigators (*cf.* 16, 17, 31, 40, 114, 116, 117, 132, 133), and in particular Kostoff (98), have reported upon certain of the same hybrids and upon 78 additional ones. On page 579 is a list of F_1 interspecific hybrids in *Nicotiana* with literature citations. In general, our findings agree with those of others who have studied the same hybrids, while the majority of such discrepancies as occur are susceptible of interpretation. In almost every instance our conclusions are based upon determinations of the extent and character of chromosome pairing at MI in from 50 to 100 pollen mother cells (PMC), all cells in a given field being independently analyzed by two investigators. In computing number of pairs where multivalents occur, a trivalent is taken to have the value of one pair, a quadrivalent of two pairs, *etc.* Meiotic behavior has been examined in plants at different stages of maturity and under different seasonal and cultural conditions. Our evidence indicates that, although certain hybrids are peculiarly sensitive to distinctions in environment, the majority do not reflect by variation of meiotic behavior such distinctions, unless they are extreme (*cf.* however, 11, 96, 102, 132). It has been reported (98) that the extent or character of pairing is different for certain reciprocal hybrids, but in our experience this is not the case.

Where reference is made to the range of chromosome pairing of a group of hybrids it will be understood that not all of the individual hybrids involved show the total inclusive range reported. Likewise, the mode may differ slightly from one hybrid to another. For example, on page 555 hybrids between members of section Tomentosae and members of sections of the other two subgenera are discussed. Chromosome behavior at MI of 18 hybrids is in-

volved, and a combined pairing range of zero to 8 with mode at 2 and at 3 is reported. These inclusive statements refer to a series of pairing ranges for individual hybrids of zero to 4, zero to 5, *etc.*, up to zero to 8, the corresponding modes being either 2 or 3 in each instance.

Twenty-eight of the 131 F_1 interspecific hybrids we have studied (50, 60, 154) and five additional ones reported by Kostoff (98) show at MI as complete or almost as complete pairing as would occur in the parental species. By contrast, another considerable group of hybrids is characterized by almost complete lack of pairing, 33 such hybrids having a range of zero to 3 (rarely 4) pairs with the mode at zero (rarely 1 or 2) pairs (50, 60). Kostoff (83, 97, 98) reports a similar range of pairing for 12 of these same hybrids and for 37 additional ones. For the 33 hybrids the mode of zero includes well over 50% of the PMC analyzed, and such lack of conjugation is taken to be a reflection of lack of genic equivalence or similarity so far as the species involved are concerned (50, p. 388). Such conjugation as occurs has been assigned to non-homologous association or autosyndesis (50, 88, 103) In 22 hybrids which we have studied and seven additional ones reported by Kostoff (83, 97, 98), there is a low but variable degree of pairing, the range in number of pairs being at times considerable (0–8). In this category the mode is consistently low with approximately 25% of the PMC showing only two or three pairs at MI. This type of pairing indicates the presence of a number of small homologous segments. In another group of hybrids (a total of 31 reported) the range in number of pairs is also wide (from one or two pairs to almost complete pairing), but the mode is higher than in the last instance and is usually at least one-half the total number of pairs possible in the particular interspecific hybrid involved, with 25% of the PMC in the modal class. The final category of pairing, where 46 hybrids have been reported, is one in which the number of pairs is the same as the haploid number of chromosomes of the parental species with the lower chromosome number, *i.e.*, "Drosera scheme" pairing (128). Obviously, it occurs only in interspecific hybrids the parents of which differ in number of chromosomes. It has been found particularly characteristic of hybrids between amphidiploid species and each of the two species the progenitors of which entered into the origin of

the particular amphidiploid. Thus, the extent of pairing exhibited by F_1 interspecific hybrids of *Nicotiana* can, in general terms, be referred to as either complete, lacking, low variable, high variable, or of the "Drosera scheme" type (*cf. N. "eastii"* p. 577).

Chromosome behavior at diakinesis is consistent with that at MI (50, 60), but knowledge of earlier prophase conditions in species as well as in hybrids is fragmentary (39, 64, 98, 102, 119). In numerous instances complete pairing at MI is followed by at least partial fertility (*cf.* 98, 114). Hybrids exhibiting other classes of pairing—low variable, high variable and "Drosera scheme" where one parent is of amphidiploid origin—are almost completely sterile, non-conjunction, non-disjunction, lagging chromosomes, *etc.*, resulting in failure of production of viable gametes (12, 98 p. 738, 103).

The quality of the pairing, as far as consistent with the karyotype patterns involved, shows correlation with its amount (50, 60). Thus, as the amount of pairing increases progressively through the categories of pairing above referred to, the chiasma frequency increases and the terminalization coefficient decreases. When there are few bivalents they are rod shaped, united by a single and frequently terminal chiasma. As the mode in number of pairs increases an increasing number of ring bivalents is seen, along with other bivalent configurations which are products of two or more chiasmata and decreased terminalization. For example, in F_1 *N. paniculata* × *N. miersii*, both species having predominantly median or submedian chromosomes and the hybrid exhibiting practically no pairing (0–2), the chiasma frequency is 1.00 per pair and the terminalization coefficient 0.85; whereas in F_1 *N. paniculata* × *N. solanifolia,* only median or submedian chromosomes and complete pairing being involved, the corresponding figures are 1.75 and 0.50, and in F_1 *N. noctiflora* × *N. petunioides,* involving all subterminal chromosomes and complete pairing, they are 1.05 and 0.60. In the example just given amount of pairing is contrasted in the first two hybrids and karyotype pattern in the last two.

In addition to bivalents, the configurations of which are reflections of chiasmatype, F_1 interspecific hybrids, particularly those of the lower pairing categories, and haploids of *Nicotiana* exhibit union of univalent chromosomes by "offspindle attachments". Two or more univalents lying at random off the MI plate region may

become attached, at times forming a chain when from three to six chromosomes are involved (125, *cf.* 105). Kostoff (98) uses the term "tandem attachment" for an apparently equivalent condition. This phenomenon has been interpreted as fixation artifact (39), persistence of the threads sometimes seen to connect univalents at diakinesis (102), as related to non-homologous pachytene association (3, 157), or residual homology (125).

Of numerous species of *Nicotiana* in which meiotic behavior has been studied, only two, "*N. eastii*" (95, 154, *cf.* p. 570) and *N. arentsii* (57), have shown multivalents at MI, and both are known to be of polyploid origin. On the other hand, Kostoff reports multivalents at MI in *N. alata* and in *N. sylvestris* (98). It is difficult to evaluate this report because the two species in question have long been under critical cytological observation by us (3, 64) and have given no evidence of valencies higher than two. Of the 131 F_1 interspecific hybrids analyzed cytologically by us (3, 50, 60, 154), approximately one-half are characterized at MI by the presence of one or more trivalents and, less frequently, higher valencies. In the hybrids he has studied Kostoff (98) refers to trivalent formation in about the same proportion of instances but rarely mentions the occurrence of higher valencies or the percentage of PMC in which multivalents are seen. Our results in this latter connection indicate that the F_1 interspecific hybrids can be classified as follows: those in which multivalents occur in from 1% to 6% of the PMC, in from 6% to 25% (most frequently 15% to 25%), in approximately 50%, and in approximately 100% of the PMC, and that these subdivisions based upon amount of multivalent formation are in general correlated with categories of extent of pairing and with degree of relationship of the species involved in the hybrids in question.

Spontaneous and induced alterations in the chromosomal content of a number of species of *Nicotiana* have been reported. Thus, for example, Mallah (109), investigating structural differences among the chromosomes of 15 varieties of *N. tabacum,* found that while the majority of them appear to be identical structurally, four varieties show structural interrelationships indicating reciprocal translocations, relative to the standard type, affecting eight chromosomes. The monosomic and trisomic condition has been shown to occur in various species (*cf.* 2, 19, 21, 24, 26, 34, 61, 64, 120, 121, 139).

Haploidy (*cf.* 86, 91, 111, 150) and chromosome doubling leading to tetraploidy, octoploidy, amphidiploidy and sesquidiploidy have frequently been reported (*cf.* 36, 37, 66, 98, 144, 145, 148, 149). Most of these categories of chromosomal alterations involving an entire chromosome complement or individual members of chromosome complements, and other conditions such as asynapsis, have been induced by treatment with high frequency radiation of reproductive and vegetative tissues of a number of species of *Nicotiana* (38, 46, 47, 51, 52, 62, 63, 64, 143). Effects of colchicine, acenaphthene, sanguinarine, centrifuging, temperature extremes, grafting and aging of seed have been shown to induce chromosome doubling, aneuploidy and structural chromosome alterations in *Nicotiana* (8, 84, 90, 92, 93, 94, 101, 138, 140, 147).

CYTOTAXONOMY

In what follows notes on the morphology and distribution of the constituent sections of the three subgenera will be supplemented by the cytological information pertaining to each of the sections with a comment, in the case of each subgenus, on the significance of the combined evidence in terms of the taxonomic arrangement (*cf.* p. 536) of the genus adopted here.

As already noted, analyses of somatic chromosome morphology in the 55 species referred to below are based upon studies of mitoses in pollen grains and root tips (50, 59, 131, 154). Meiotic chromosome behavior at MI in some 210 F_1 interspecific hybrids is also discussed. Reference has already been made (p. 540) to the character and quality of pairing in these hybrids and their parental species, to the correlation between chiasmatype and karyotype and to the chiasma frequencies and terminalization coefficients of the three subgenera. Although species the close relationship of which is otherwise variously indicated commonly show a high degree of compatibility, the possibility of securing a mature F_1 hybrid between *Nicotiana* species is not a reliable index of relationship. It has been reported that compatibility shows "greater relationship to chromosome number than to taxonomic status" (16, 114), that its extent indicates "the trend of relationship in a general way" (34), and that "crossibility decreases with decrease in relationship" (98). However, many F_1 hybrids which earlier investigators failed to obtain have since been successfully made. There have

been efforts to set up categories of compatibility (16, 34, 98, 110, 112). It has been shown that in *Nicotiana,* as elsewhere, crossibility is related to the use of the species possessing the higher chromosome number as female parent, although there are notable exceptions (34, 50, 83, 98). In certain cases seasonal effects, mechanical conditions and stage of maturity are said to influence crossibility (35, 83, 98). Undoubtedly of significance is the possibility of genic blocks to successful hybridization. In this general connection attention is called to the fact that the appended list of F_1 interspecific hybrids in *Nicotiana* includes only those for which cytological evidence is available. It is, therefore, in no sense an index of compatibility, since numerous additional hybrids have been obtained (*cf.* 110, 112). References to the literature concerning many of the hybrids discussed here are given in the list only. The morphology of the majority of the hybrids referred to has been described in considerable detail (67, 98, 124).

Weight is assigned to the extent and quality of pairing at MI of interspecific hybrids in interpretation of relationships between species and larger taxonomic subdivisions (50 p. 388). Meiotic behavior and categories of pairing in hybrids have already been discussed (*cf.* p. 542). The fact that the phylogenetic conclusions reflected in the present taxonomic arrangement are supported by evidence from morphology, distribution and karyotype pattern as well as by extent of pairing in F_1 interspecific hybrids should be borne in mind in connection with a statement by Darlington (29 p. 172) which gives the impression that pairing relations alone have been used as a criterion in establishing species origins and relationships in the genus *Nicotiana.*

Subgenus Rustica

Section Paniculatae: *N. paniculata, N. knightiana, N. solanifolia, N. benavidesii, N. raimondii, N. cordifolia, N. glauca.*

Section Thyrsiflorae: *N. thyrsiflora.*

Section Rusticae: *N. rustica.*

Stout herbs or short-lived shrubs with yellow or green, tubular or tubular-salverform corolla.

This subgenus represents, in general, a morphological unit. Vegetatively, it presents two patterns: (a) a relatively thick stemmed, erect, bushy herb or shrub type provided with well

spaced, petioled leaves (Paniculatae and Rusticae); (b) a spicate plant type clothed with crowded, narrow, sessile leaves (Thyrsiflorae).

Three flower types that correspond to the three sections are distinguishable on the basis of corolla shape and stamen characters—those of the first and last sections less distinct from each other, and that of the Thyrsiflorae more distinct than those of the other two. In the Paniculatae all six species are clearly inter-related; *N. paniculata* and *N. knightiana* show close morphological affinity in the majority of characters, while resemblance in flower relates *N. raimondii* to *N. cordifolia* and *N. solanifolia* to *N. benavidesii*. In leaf shape and in habit (exclusive of inflorescence) *N. benavidesii* approaches *N. glutinosa* of subgenus Tabacum, section Tomentosae. The structure of the inflorescence together with longer life span and greater woodiness sets *N. glauca* somewhat apart from the other members of the Paniculatae.

Five of the nine species of this subgenus occur only in Peru, and for a sixth, *N. rustica*, which is also known from Ecuador, the Peruvian Andes represent the center of distribution. All of these six species occupy arid or semi-arid habitats, but only *N. paniculata* is widespread in latitudinal distribution (near the coast), while *N. thyrsiflora* is restricted to the northern highlands, *N. benavidesii* and *N. raimondii* to the southern Andes, and *N. knightiana* to the southern coast. Of the remaining three species in the subgenus, *N. cordifolia* is endemic on Mas-a-Tierra Island in the Juan Fernandez group west of central Chile, *N. solanifolia* has a limited distribution in the fog belt on the coast of northern Chile and *N. glauca*, although introduced and now widely distributed elsewhere, is found in the wild state only in northwestern Argentina. The first two of these geographically outlying species possess considerable morphological resemblance to all the remaining species except *N. thyrsiflora* and *N. glauca*. The last named reflects something of a morphological hiatus so far as the subgenus as a whole is concerned.

Section Paniculatae. a. Karotypes. Each of the seven species of this section—*N. glauca, N. raimondii, N. solanifolia, N. cordifolia, N. benavidesii, N. knightiana, N. paniculata* (in order of decreasing average chromosome size)—possesses 12 pairs (48, 50, 59). Except in *N. glauca* and *N. benavidesii*, all the chromosomes of these

species are median or submedian, and in each species one submedian pair is satellited. *N. benavidesii* conforms to this basic pattern except that its karyotype contains two subterminal pairs, one satellited (59). The detailed formulae for six of the species of this section are: *N. raimondii*, *N. solanifolia* and *N. cordifolia*— 4 *m* + 8¹ *sm*; *N. knightiana* and *N. paniculata*—6 *m* + 6¹ *sm*; *N. benavidesii*—6 *m* + 4¹ *sm* + 2¹ *st* (the figure referring to the number of pairs, the superscript designation to the number of satellited pairs, and *m* and *sm* to median or submedian centromere position). By contrast with this general uniformity of karyotype pattern, most of the chromosomes of *N. glauca* are subterminal with the formula 1 *m* + 1 *sm* + 10¹ *st* (50, 59).

One other study of *N. glauca* (131) made of root tip mitoses only, provides the formula 1 *m* + 11¹ *st*. The chromosomes of *N. glauca* have frequently been reported to be the longest in the genus (16, 50, 98, 131). The rod-like form of most of the *N. glauca* genom creates such an impression, whereas actual measurement shows that the total chromosome length in *N. raimondii* or *N. solanifolia* approximates, and in *N. acaulis* (subgenus Petunioides, section Noctiflorae) considerably exceeds, the total length in *N. glauca*.

    b. Meiotic chromosome behavior in F₁ interspecific hybrids. As shown in the subjoined list (p. 579) which, as already noted, refers only to F₁ interspecific hybrids which have been studied cytologically, both *N. raimondii* and *N. solanifolia* have been crossed with all other species of the Paniculatae and *N. benavidesii* and *N. paniculata* with all except *N. cordifolia*. With the last named *N. knightiana* has been crossed, and if, as appears certain, *N. paniculata* and *N. knightiana* are practically equivalent genically, the series of F₁ intrasectional hybrids includes all possible combinations of the species concerned, except those involving *N. glauca*. With the exception of F₁ *N. benavidesii* × *N. solanifolia* which, however, shows high variable pairing with a range of 2 to 11 pairs and a mode at 6 pairs, chromosome conjugation is complete or nearly so at MI of these nine hybrids. In all except those involving *N. cordifolia* multivalents occur in from 12% to 25% of the PMC.

With the exception of *N. cordifolia*, *N. glauca* has been crossed with every other species of the section. In each case pairing is lacking or of the low variable type. For example, in F₁ *N. panicu-*

lata × *N. glauca* the pairing range is zero to 3 with the mode at zero pairs.   In this case Kostoff (83) reported a range of 9 to 12 pairs and of 6 to 10 pairs for the reciprocal and, later (98), a range of 4 (rarely 3) to 10 pairs and some trivalents for the original hybrid.

Between members of the Paniculatae and members of the other two sections of subgenus Rustica and also members of the other two subgenera, F_1 hybrids have been made.   Within the subgenus, *N. benavidesii, N. paniculata* and *N. solanifolia,* when crossed with *N. rustica,* show "Drosera scheme" pairing (*cf.* p. 551) with considerable multivalent formation.   For F_1 *N. rustica* × *N. glauca* an approximation of "Drosera scheme" behavior has been reported (98 p. 675) in the sense that 12 pairs are "very rarely formed" (98 p. 677).   Low variable pairing occurs in F_1 *N. thyrsiflora* × *N. glauca* (*cf.* p. 550).   As a genically representative species of all the members of its section (*N. glauca* excepted), *N. paniculata* has been crossed with at least one member of each of the eight sections of the other two subgenera, except the Trigonophyllae and the Suaveolentes (*N. tabacum, N. glutinosa, N. undulata, N. noctiflora, N. miersii, N. langsdorffii, N. bigelovii, N. alata, N. sanderae*). We have studied all but the last three of these hybrids, and meiotic behavior in every instance is irregular and pairing is either lacking or of the low variable category.   According to this evidence the reports by Kostoff (98) of pairing ranges from 2 to 7 for F_1 *N. paniculata* × *N. alata* and × *N. sanderae,* from 3 to 8 for F_1 *N. paniculata* × *N. langsdorffii,* for which he previously (83) reported zero to 7 pairs (*cf.* also 16, 31), and from 2 to 12 for F_1 *N. paniculata* × *N. tabacum* are difficult to understand.   Four F_1 hybrids between species of the Paniculatae and members of subgenus Tabacum (*N. benavidesii, N. raimondii* and *N. solanifolia* with *N. tabacum* and *N. benavidesii* × *N. glutinosa*) show low variable pairing, while two hybrids involving members of subgenus Petunioides, F_1 *N. solanifolia* × *N. suaveolens* (section Suaveolentes) and F_1 *N. solanifolia* × *N. bigelovii* (section Acuminatae) show lack of pairing.

In the F_1 hybrids of *N. glauca* with *N. noctiflora* of section Noctiflorae, with *N. nudicaulis,* with *N. bigelovii* and with *N. attenuata,* three species of section Acuminatae, subgenus Petunioides, the author has observed a negligible amount of pairing.   Kostoff (98)

has studied the first three of these hybrids and his results are in agreement with ours. He reports similar pairing for F$_1$ *N. glauca* × *N. suaveolens* and × *N. megalosiphon* of section Suaveolentes. Present evidence indicates (60) a pairing range of zero to 8 pairs (most frequently 4 or 5) for F$_1$ *N. tabacum* × *N. glauca*, whereas Sarana (133) finds "up to 12 pairs" and Kostoff (98) reports 9 to 12 pairs. Similarly, the latter investigator (98) for F$_1$ hybrids between *N. glauca* and three other species of subgenus Tabacum (*N. tomentosa*, *N. glutinosa* and *N. tomentosiformis*) reports high pairing, and an approximation of "Drosera scheme" pairing for hybrids between *N. glauca* and five members of section Alatae, subgenus Petunioides (*N. alata*, *N. langsdorffii*, *N. sanderae*, *N. longiflora* and *N. plumbaginifolia*). These results are difficult to reconcile and are subject to review, especially since others find a negligible amount of pairing in F$_1$ *N. glauca* × *N. plumbaginifolia* (125), and the author has found little pairing in *N. glauca* × *N. tomentosa*.

Section Thyrsiflorae. This section consists of one 12-paired species, *N. thyrsiflora*. A difficult plant to grow to maturity, it has proved impossible thoroughly to analyze its karyotype or investigate its relationships with other species. Nevertheless, it is clear that like all species of the Paniculatae except *N. glauca*, all its chromosomes are median or submedian, one pair being satellited (59). The one interspecific hybrid made, F$_1$ *N. thyrsiflora* × *N. glauca*, shows at MI a range of zero to 5 pairs, with the mode at 1 pair (60).

Section Rusticae. Here also the section consists of a single species, *N. rustica*. The only species of the subgenus possessing 24 pairs (122), it has been shown to be an amphidiploid, progenitors of members of the Paniculatae (presumably *N. paniculata*) and of *N. undulata* (subgenus Petunioides, section Undulatae), having entered into its origin by hybridization followed by chromosome doubling (18, 50, 66, 89, 98, 100, 103, 104).

    a. *Karyotype*. From study of root tip mitoses the karyotype of *N. rustica* has been reported to consist of chromosomes without morphological distinctions (130) and small and sharply flexed (15), uniform except for the presence of two subterminal pairs with one pair bearing proximal satellites (131), and very similar to *N. paniculata* chromosomes (89). Detailed analysis of chromo-

some morphology in pollen grain mitoses (59) shows 12 m and 12^2 sm with only slight range in size, the average length being somewhat less than that of the $N.$ $paniculata$ complement which contains the smallest chromosomes of all the members of the Paniculatae (50, 59).

   b. Meiotic chromosome behavior in F_1 interspecific hybrids. As already noted (*cf.* p. 549), F_1 hybrids between $N.$ $rustica$ and three members of the Paniculatae ($N.$ $paniculata,$ $N.$ $benavidesii$ and $N.$ $solanifolia$) exhibit "Drosera scheme" pairing, most complete in the hybrid involving $N.$ $paniculata.$ Equivalent pairing with considerable multivalency obtains in F_1 $N.$ $rustica \times N.$ $undulata$ of subgenus Petunioides. In hybrids of $N.$ $rustica$ with $N.$ $alata,$ $N.$ $palmeri,$ $N.$ $sanderae,$ $N.$ $langsdorffii$ and $N.$ $glauca$ (*cf.* pp. 549, 566), Kostoff (98) reports high variable pairing with some multivalency. By contrast, in hybrids of $N.$ $rustica$ with one or more members of the Undulatae, Acuminatae, Noctiflorae and Suaveolentes of subgenus Petunioides ($N.$ $wigandioides,$ $N.$ $noctiflora,$ $N.$ $pauciflora,$ $N.$ $miersii,$ $N.$ $attenuata,$ $N.$ $bigelovii,$ $N.$ $nudicaulis,$ $N.$ $suaveolens$), and in F_1 $N.$ $rustica \times N.$ $tabacum$ (subgenus Tabacum) there is lack of pairing or low variable pairing in every case (*cf.* pp. 556, 562, 567, 569, 573). In other words, only four of 18 F_1 interspecific hybrids involving $N.$ $rustica$ consistently show appreciable pairing at MI, and in those four "Drosera scheme" pairing occurs. Therefore it appears that the gametic complement of 24 $N.$ $rustica$ chromosomes, which in the haploid individual shows no pairing (80), consists of two subgenoms of 12 each, one derived originally from a progenitor of $N.$ $paniculata$ or of its close modern relatives and the other from a progenitor of $N.$ $undulata.$ Confirmation of this hypothesis was obtained by the production of fertile, 24-paired $N.$ $rustica$-like plants from unions of unreduced male gametes of F_1 $N.$ $undulata \times N.$ $paniculata$ with normal female gametes of $N.$ $rustica$ (50).

   Comment. The Paniculatae contains seven species the intimate relationship of six of which, indicated by their morphology and distribution, is confirmed by their common karyotype pattern, their extensive compatibility and the complete or nearly complete pairing that their F_1 hybrids exhibit. The karyotype of $N.$ $glauca$ and pairing in F_1 hybrids involving this species represent an exception to the uniformity in cytological detail characteristic of the section in which

it is placed and of the subgenus as a whole. Since, however, its morphological expression is essentially in accord with that of other members of the Paniculatae, it has provisionally been retained there. Because of certain morphological deviations and the distributional and cytological evidence, there may, on the other hand, be sufficient justification for placing *N. glauca* in a section by itself. In the Thrysiflorae, although confirming cytological evidence is meager, the morphological distinctions between *N. thyrsiflora* and members of the Paniculatae justify the separation of this species under a sectional designation, at least until chromosome behavior in inter-specific hybrids involving it has been determined. On certain morphological and distributional grounds *N. rustica* might be con-sidered a member of the Paniculatae, one species of which has apparently entered into its amphidiploid origin. However, it is placed in a distinct section in part because the other parent involved in such origin is a member of a different subgenus.

Subgenus Tabacum

Section Tomentosae: *N. tomentosa, N. tomentosiformis, N. oto-*
     phora, N. setchellii, N. glutinosa.
Section Genuinae: *N. tabacum.*

Stout herbs or limited perennial shrubs with showy, white, red-dish or yellowish flowers, the gaping corolla throat expanded into a cup, the stamens more or less exserted.

This subgenus consists of a nucleus of three closely related species (*N. tomentosa, N. tomentosiformis, N. otophora*) from which, and from one another, the three remaining species are somewhat sepa-rated morphologically. The first three are few- to many-stemmed perennials (or limited perennials), subarborescent in height, habit and deciduous character of the lower leaves, with a distinct inflo-rescence axis, little or no branching of the lateral elements of the inflorescence, and the throat cup of the corolla distinct. *N. to-mentosa* is highly polymorphic, *N. otophora* less so; *N. tomentosi-formis* has closest affinity with *N. tomentosa* but approaches *N. otophora*. *N. setchellii* is practically identical with the preceding three species in leaf character, rather similar in habit and inflor-escence, least similar in its subcampanulate flower. *N. glutinosa* exhibits the flower type of the first three species but differs from them in inflorescence, leaf and life span. The remaining species of

the subgenus, *N. tabacum*, is exceedingly polymorphic. It is morphologically reminiscent of *N. tomentosa*, *N. tomentosiformis* and *N. otophora* but rarely is characterized by either their woodiness, longevity, subarborescence, relatively simple inflorescence pattern or highly specialized flower. In shape the corolla represents a compromise between the cup-throated and salverform types, varying at times in one direction or the other.

The distribution of the subgenus is essentially continuous and restricted, largely Andean. *N. tomentosa* is found from a little north of central Peru to western Bolivia. *N. glutinosa* shows a range from the coast of the northern half of Peru along the western flank of the outer Cordillera into southern Peru, while *N. setchellii* is apparently restricted to north Andean Peru. Although often giving the appearance of a naturally occurring species in the lower Andes from northern Peru to northern Argentina, *N. tabacum* is there probably only an escape from cultivation and, otherwise, has no known present day distribution except as a crop plant or relic of cultivation. *N. otophora* is found in the Yungas of central Bolivia and the subtropical zone of northwestern Argentina; *N. tomentosiformis* in western Andean Bolivia. Of the species of the subgenus Tabacum, *N. glutinosa* alone is characteristic of warm, arid areas, and in the Andes tends to occupy the region in which occurs *N. benavidesii*, the species of the subgenus Rustica with which it has characters in common. All other species are usually restricted to deep valleys in which they seek sheltered and well lighted situations. Their habitat is, therefore, temperate to semi-tropical and even at times marginal in the moisture-laden *ceja de la montaña*.

Section Tomentosae. a. Karyotypes. Of the five 12-paired species composing this section, the three which on morphological grounds are most closely related (*N. tomentosa*, *N. tomentosiformis*, *N. otophora*) possess the common karyotype pattern $7^1 m$ or $sm + 5^1 st$, while for *N. setchellii* and *N. glutinosa* it is $12^1 m$ or sm (50, 59). Of the first three species, *N. tomentosiformis* and *N. otophora* are practically identical in chromosome morphology. Both complements consist of seven small median chromosomes and five subterminal ones, the latter three to four times the length of the former. Such a strikingly dimorphic genom has no parallel elsewhere in the genus. The genom of *N. tomentosa* is also somewhat dimorphic with four submedian, three median and

five subterminal pairs, but the entire complement is smaller and size distinctions between the subterminal and the median or submedian pairs are not striking. Like *N. tomentosiformis* and *N. otophora*, *N. tomentosa* has one median and one subterminal pair satellited. In *N. tomentosa* one pair of each of the three types of chromosomes shows an additional constriction (*cf.* p. 540) not apparent in the two other species. An earlier analysis (50) of the genom of *N. tomentosiformis*, made of root tip mitoses only, does not entirely agree with the above characterization of its karyotype which is based upon studies of pollen grain mitoses also (59).

As noted above, in *N. glutinosa* and *N. setchellii* a different karyotype pattern occurs—12^1 *m* or *sm* (59); in *N. glutinosa* 5 *m* and 7^1 *sm*, in *N. setchellii* 7^1 *m* and 5 *sm*. The chromosomes of these two species show no marked distinctions in length. The *N. glutinosa* genom has been reported to consist of chromosomes uniform in size and shape (16), of median and submedian ones of seven types in terms of relative size, length of arms and presence of a satellite (131), and Webber (150), in the case of haploid plants, found nine median or submedian and three subterminal chromosomes. Additional evidence supports the karyotype formula first noted above, which is the one earlier reported by the author (50). In three submedian pairs the short arm is reduced but never to one-fourth the total length of the chromosome involved.

b. Meiotic chromosome behavior in F_1 interspecific hybrids. Each of the first four members of the Tomentosae has been crossed with all the others, and meiotic chromosome behavior studied. In F_1 *N. tomentosiformis × N. otophora* it could not be determined because of early degeneration of anther tissue. In each of the other five F_1 hybrids (*cf.* appended list of hybrids, p. 579) chromosome conjugation at MI is complete or practically so. The fifth member, *N. glutinosa*, has been crossed with all other species of the Tomentosae except *N. setchellii*, and according to my evidence pairing in these three F_1 hybrids is of the low variable type with a range of zero to 9 pairs, mode at 3 or at 5, with some multivalent formation (50, 60). For two of these hybrids—F_1 *N. glutinosa × N. tomentosa* and × *N. tomentosiformis*—Kostoff (98) reports a pairing range of 2 to 11 (most frequently 6 to 8) and 3 to 10 (most frequently 5 to 8), respectively, and some multivalency in both.

Hybrids between all members of the two sections of the subgenus

have been studied cytologically. In F_1 *N. tabacum* $\times$ *N. glutinosa* considerable variation in amount of pairing occurs (*cf.* p. 556), whereas all the other F_1 hybrids involving *N. tabacum* and members of the Tomentosae show "Drosera scheme" pairing, with considerable multivalent formation.

One or more species of section Tomentosae has been crossed with members of the following sections of the other two subgenera: Paniculatae (*N. glauca, N. benavidesii, N. paniculata*); Alatae (*N. sylvestris*); Undulatae (*N. undulata, N. wigandioides*); Acuminatae (*N. bigelovii, N. nudicaulis*); Trigonophyllae (*N. trigonophylla, N. palmeri*); Suaveolentes (*N. suaveolens*). Most of these 25 F_1 hybrids exhibit low variable pairing with a range of zero to 8 and mode at 2 or at 3 pairs (50, 60, 98). In F_1 *N. glutinosa* $\times$ *N. wigandioides* (the Undulatae), the author has found a range of 1 to 9 with mode at 6 pairs, and Elvers (originally reporting the hybrid as *N. glutinosa* $\times$ *N. tomentosa*) found 2 to 9, most frequently 4 to 6, pairs (39). In the case of two hybrids involving section Trigonophyllae the amount of pairing is consistently greater —F_1 *N. trigonophylla* $\times$ *N. tomentosa* showing 2 to 10 or 11 pairs with the mode at 5 or 7 pairs (50, 98), and F_1 *N. trigonophylla* $\times$ *N. setchellii* 2 to 10, mode 5 (60). Considerable variability in amount of pairing in different PMC under varying environmental conditions was seen in F_1 *N. palmeri* $\times$ *N. setchellii*, from low variable (0–7) to high variable (5–11) and frequent trivalency (60). In three F_1 hybrids, *N. glauca* $\times$ *N. glutinosa*, $\times$ *N. tomentosa* and $\times$ *N. tomentosiformis*, Kostoff (98) has found high pairing (3–10, 8–11 and 8–12 respectively) with some trivalency. In the only one of these hybrids that the author also has studied almost no pairing was seen (*cf.* p. 550).

Section Genuinae. a. Karyotype. In the amphidiploid origin of the single species of this section, *N. tabacum*, two species, each with 12 pairs of chromosomes, were concerned, and thus its genom shows 24 pairs. Ten of them are median or very nearly median, five are submedian and nine subterminal (59), one pair of each of these three morphological categories bearing proximal satellites ($10^1 \, m + 5^1 \, sm + 9^1 \, st$). Of the ten median pairs, three are conspicuously long and more or less sharply flexed at the centromere as contrasted with the remaining seven which are exceedingly short and generally only slightly flexed (59). The longest pair with

arms of almost equal length, as well as two additional pairs of submedian chromosomes, have conspicuous constrictions (*cf.* p. 540) near the extremities of the longer arms. The subterminal chromosomes show little variation in length, the longest being approximately as long as the longer median ones. In root tips Ruttle (129a) notes conspicuous distinctions in chromosome size and morphology in the *N. tabacum* genom and reports two satellited pairs. Sarana (131) refers to ten chromosome types on the basis of differences in size, position of centromere and presence or absence of satellites. By other investigators the *N. tabacum* karyotype has been described as consisting of chromosomes shorter and thicker than those of *N. rustica* (118), larger and not so sharply flexed as those of *N. rustica* (15), and showing considerable individual size distinctions (130).

b. Meiotic chromosome behavior in F_1 *interspecific hybrids.* As already noted, F_1 hybrids made between *N. tabacum* and every other member of its subgenus except *N. glutinosa,* show "Drosera scheme" pairing, with frequent multivalency. The F_1 hybrid *N. tabacum* × *N. glutinosa* has been studied by a number of investigators who have variously reported: an approximation of "Drosera scheme" behavior (25), lack of pairing (17), low variable pairing with the mode at 2 (50, 124) or at 4 (117) pairs. More recently, Kostoff (98) refers to a highly variable number of pairs with the most frequent range between 2 and 6, while the author's analyses (60) show the extremely wide range of zero to 11 pairs with the mode at 5 and some multivalency.

An extensive series of F_1 hybrids between *N. tabacum* and six members of subgenus Rustica (*N. raimondii, N. paniculata, N. benavidesii, N. solanifolia, N. glauca, N. rustica*), and eleven members of subgenus Petunioides (*N. sylvestris, N. suaveolens, N. debneyi, N. palmeri, N. bigelovii, N. nudicaulis, N. acuminata, N. pauciflora, N. alata, N. sanderae, N. longiflora*) have been analyzed cytologically. We have studied most of these F_1 hybrids, and our evidence indicates that in every case, with the exception of F_1 *N. tabacum* × *N. sylvestris* where "Drosera scheme" behavior obtains, they fall into the two categories of least pairing (*cf.* also p. 557).

Comment. Like subgenus Rustica and in terms of morphology, distribution, karyotype pattern and extent of pairing at MI and F_1

intra- and intersubgeneric hybrids, subgenus Tabacum has been shown to have distinct identity and to consist of (*a*) a core or nucleus of closely related species, (*b*) related but somewhat outlying species, and (*c*) an amphidiploid species in the origin of which, as will be shown below, progenitors of members of the subgenus in question and of a member of another subgenus were concerned.

Comparable to the position of *N. glauca* in the Paniculatae, is that of *N. glutinosa* in the Tomentosae. Less of the cytological but more of the morphological evidence than in the case of *N. glauca* might justify the separation of *N. glutinosa* under a sectional designation.

In the case of *N. tabacum* and its numerous F_1 interspecific hybrids much cytological evidence is available, the majority of which bears upon the nature of the amphidiploid origin of this, the most important, tobacco plant of commerce (33, 70, 123). As indicated above, the *N. tabacum* genom of 24 chromosomes consists of two subgenoms of 12 chromosomes each which differ from each other in karyotype pattern. The chromosome morphology of one subgenom conforms to that of the three species forming the nucleus of section Tomentosae, that of the other subgenom to the chromosome morphology of *N. sylvestris* (subgenus Petunioides, section Alatae). Pairing at MI in F_1 hybrids involving *N. tabacum* is, according to the author's evidence, of one of two types: (*a*) "Drosera scheme", but only in the case of hybrids made either with *N. sylvestris* or with the nucleus species of section Tomentosae, or (*b*) low variable (0–8, with mode at 2, 3 or 4 pairs). Analyses of the same hybrids by others are confirmatory in the first but in certain cases contradictory in the second instance. Thus, Kostoff (98) reports high variable, and rarely "Drosera scheme", pairing in F_1 *N. tabacum* × *N. paniculata*, × *N. glauca*, × *N. sanderae*, × *N. alata* and × *N. longiflora*. For the hybrid between the two amphidiploid species *N. tabacum* and *N. rustica*, "only a few loose bivalents" (16, 144) or zero to 7 pairs with a mode at 3 pairs (50, 60) have been found, while Kostoff reports 5 to 24 (98). Reexamination of our evidence fails to indicate the cause of the discrepancies between our results and those of Kostoff.

The significance of the foregoing cytological information for interpretation of the origin of *N. tabacum* is as follows. Winge's hypothesis (155, 156) that certain species have had their origin

through interspecific hybridization followed by chromosome doubling was verified in the case of *N. "digluta"* (25) which originated by such doubling in F_1 *N. tabacum* × *N. glutinosa*.   The proposal was then advanced (70) that *N. tabacum* had a similar origin, progenitors of *N. sylvestris* and of *N. tomentosa* (or *N. tomentosiformis*) being initially involved (*cf.* also 77).   The cytological evidence that F_1 *N. tabacum* × *N. sylvestris,* × *N. tomentosa* and × *N. tomentosiformis* (*cf.* 9, 10, 20, 69, 70, 83) show "Drosera scheme" pairing and that pairing is largely lacking in F_1 *N. sylvestris* × *N. tomentosa* and also in haploid individuals of *N. tabacum* (*cf.* 14, 27, 70, 78, 102, 111), furnishes a background for this proposal.   It is now known (60, 66) that *N. setchellii* and *N. otophora* crossed with *N. tabacum* also exhibit "Drosera scheme" pairing, and for this reason and because of present day overlapping in the ranges of distribution of *N. sylvestris* and *N. otophora* (56), and also on morphological grounds, it has been suggested that a progenitor of *N. otophora* rather than of *N. tomentosa* or *N. tomentosiformis* was concerned in the origin of *N. tabacum* (56, 66).   Confirming evidence of the nature of its amphidiploid origin is derived from its dual karyotype constitution and from efforts to synthesize *N. tabacum* either from trigenomatic hybrids involving *N. sylvestris* and members of the Tomentosae (5, 6, 9, 10, 85, 108) or by artificially induced chromosome doubling in appropriate F_1 hybrids (75, 76, 77, 94).   The individuals so produced resemble *N. tabacum* morphologically and show 24 pairs at MI but are largely sterile (22). Especially significant are the analyses of monosomic types in *N. tabacum* when crossed with *N. sylvestris*.   Clausen and Cameron obtained all of the possible 24 monosomics (23) and have succeeded in classifying each of them as monosomic either for a member of the *N. sylvestris* or of the *N. tomentosa* subgenom of *N. tabacum*. In the 35-chromosome F_1 hybrids of monosomic *N. tabacum* crossed with *N. sylvestris* the MI pairing is 12 bivalents + 11 univalents if the *N. tabacum* parent is monosomic for a member of the *N. tomentosa* subgenom, or 11 bivalents + 13 univalents if it is monosomic for a member of the *N. sylvestris* subgenom.   Twelve of the monosomic *N. tabacum* types were found to fall into one and twelve into the other of the two pairing categories when crossed with *N. sylvestris.*

Subgenus Petunioides

Section Undulatae: *N. undulata, N. wigandioides, N. arentsii.*
Section Trigonophyllae: *N. trigonophylla, N. palmeri.*
Section Alatae: *N. alata, N. langsdorffii, N. bonariensis, N. longi-
flora, N. plumbaginifolia, N. sylvestris, N. repanda, N. stock-
tonii, N. nesophila.*
Section Noctiflorae: *N. noctiflora, N. petunioides, N. ameghinoi,
N. acaulis.*
Section Acuminatae: *N. attenuata, N. acuminata, N. pauciflora, N.
corymbosa, N. miersii, N. linearis, N. spegazzinii, N. cleve-
landii, N. bigelovii, N. nudicaulis.*
Section Suaveolentes: *N. suaveolens, N. maritima, N. velutina, N.
fragrans, N. megalosiphon, N. gossei, N. excelsior, N. ingulba,
N. goodspeedii, N. exigua, N. rotundifolia, N. occidentalis, N.
stenocarpa, N. debneyi, N. benthamiana.*

Annual, rarely perennial, herbs with the corolla almost always
white and salverform.

This subgenus is not only larger but considerably more hetero-
geneous morphologically than the preceding two subgenera. Most
typical are the four sections Alatae, Noctiflorae, Acuminatae and
Suaveolentes. In practically all instances their species are vesper-
tine and are characterized by a rosette, often of considerable size
and duration, as contrasted with the remainder of the genus in
which the flowers remain expanded in sunlight and which develop
little or no rosette. The Alatae, Noctiflorae and Acuminatae have
distinct identity, and the essential members of any one of these three
sections would not readily be confused with those of either of the
other two. The species of the Suaveolentes, a geographically iso-
lated group, are composites of the morphologically distinguishing
characters of the three other sections to which reference has just
been made.

In the Undulatae certain subgenus Rustica characters are recog-
nizable in *N. undulata,* and certain subgenus Tabacum characters
in *N. wigandioides,* while *N. arentsii* combines major morpholog-
ical elements of these two species. Thus, section Undulatae rep-
resents a bridge or transition between subgenera and is therefore
placed first in the sequence of sections. Section Trigonophyllae is
clearly a member of subgenus Petunioides, although the flower of
N. trigonophylla is reminiscent of that of species of the Paniculatae

in subgenus Rustica. Rarely, there is occurrence of characters of the latter subgenus elsewhere in subgenus Petunioides as, for example, in *N. langsdorffii* of the Alatae. Species especially closely related morphologically in subgenus Petunioides are: *N. longiflora* and *N. plumbaginifolia; N. acuminata* and *N. pauciflora; N. trigonophylla* and *N. palmeri; N. corymbosa* and *N. linearis; N. repanda, N. stocktonii* and *N. nesophila; N. maritima* and *N. velutina; N. rotundifolia* and *N. occidentalis; N. gossei* and *N. excelsior.* Species most divergent, either florally or vegetatively, from the other species in their respective sections are: *N. langsdorffii, N. sylvestris,* the *N. repanda-N. stocktonii-N. nesophila* group, *N. bigelovii,* and *N. benthamiana.*

By contrast with the strictly South American range of natural distribution of species of the subgenera Rustica and Tabacum, that of members of subgenus Petunioides includes North America, Australia and the South Pacific as well as South America. Two sections (Noctiflorae and Undulatae) are exclusively South American, another (Trigonophyllae) exclusively North American, a fourth (Suaveolentes) is confined to Australia and certain of the South Pacific islands, while the two remaining sections (Alatae and Acuminatae), although largely South American, have representatives in North America also. Apart from the Undulatae (Andes of Peru and Bolivia), the South American species of subgenus Petunioides occur south of the Tropic of Capricorn and therefore below the distributional range of the species of the two other subgenera. Thus, the Noctiflorae occur in western central Argentina, the center of distribution of the South American species of the Alatae and the Acuminatae is the Brazilian-Uruguayan border and central Chile, respectively, with the ranges of all three sections overlapping in northern Argentina and those of the Noctiflorae and the Acuminatae in Patagonia. In American distribution *N. longiflora* and *N. plumbaginifolia* of the Alatae and *N. acuminata* of the Acuminatae range most widely. The presence of *N. longiflora* at several coastal stations in eastern and southeastern United States and of *N. acuminata* in California and Washington is presumably due to man's intervention. It is doubtful that the same agency is responsible for the distribution of *N. plumbaginifolia* from southeastern Brazil to northwestern Peru and its reappearance in southern Mexico and northward along both Mexican coasts

and in the West Indies. Three other species of the Alatae are found in North America and exclusively there: *N. repanda,* in northeastern Mexico, *N. stocktonii* and *N. nesophila,* endemics of the Revillagigedo Islands, southwest of Lower California. Similarly, in the Acuminatae there is a species (*N. acuminata*) found in both Americas, while three species are exclusively North American: *N. bigelovii* chiefly in California, *N. clevelandii* in southern California and Lower California, *N. nudicaulis* in northeastern Mexico.

A considerable morphological diversity within subgenus Petunioides is paralleled by diversity in the habitats of its constituent species. With the exception of *N. undulata,* a subalpine species, the members of the Undulatae and the Alatae tend to occupy subtropical situations, some more moist and others somewhat drier. The Trigonophyllae, Noctiflorae and Acuminatae are peculiar to dry habitats (deserts, high Andean plateaus, the Patagonian steppe). Many species of the Suaveolentes are characteristic of arid or semi-arid areas, interior and coastal; a few in the Australian deserts only. Rainfall is greater at certain of the coastal and at the insular stations.

Section Undulatae. a. Karyotypes. The two 12-paired species of the Undulatae differ in karyotype pattern: *N. undulata* has $6 m + 6^1 sm$, *N. wigandioides* $5 m + 3 sm + 4^1 st$, with no marked size difference within each genom nor between the two genoms (57, 59). Sarana's report (131) of all subterminal chromosomes in *N. undulata* was undoubtedly due to error in identification of the species (98, p. 85). The karyotype of 24-paired *N. arentsii* (11 $m + 9^1 sm + 4^1 st$) reflects completely the karotypes of *N. undulata* and *N. wigandioides,* the two species which entered into its amphidiploid origin (57, 59). In this connection it is of interest that at MI of *N. arentsii* certain particular bivalent configurations characteristic of MI in the parent species can be recognized (57).

b. Meiotic chromosome behavior in F_1 interspecific hybrids. The cytology of the three F_1 hybrids between the members of the Undulatae has been determined (57). F_1 *N. undulata* × *N. wigandioides* exhibits high variable pairing (2–9, mode 5). In F_1 *N. arentsii* × *N. undulata* and × *N. wigandioides* "Drosera scheme" pairing occurs with extensive multivalency, the modal class in each hybrid including a trivalent.

By contrast, among F_1 hybrids of *N. undulata* with three mem-

bers of other sections of subgenus Petunioides (*N. longiflora* and
N. langsdorffii of the Alatae and *N. attenuata* of the Acuminatae),
with five members of subgenus Rustica (*N. rustica, N. paniculata,
N. raimondii, N. benavidesii* and *N. cordifolia*), and with one
member of subgenus Tabacum (*N. glutinosa*), all except one ex-
hibit almost complete lack of pairing or pairing of the low variable
type. This exception is F_1 *N. rustica* × *undulata* where "Drosera
scheme" pairing with a high degree of multivalency occurs (*cf.* p.
551). *N. wigandioides* crossed with *N. rustica* exhibits low vari-
able pairing and with *N. glutinosa,* subgenus Tabacum (*cf.* p. 555),
only a slightly higher degree of pairing.

   Section Trigonophyllae. a. Karyotypes. The karyotype for-
mula of 12-paired *N. trigonophylla* is $4\ m + 5\ sm + 3^1\ st$ (50, 59).
Composed of uniformly small chromosomes, the genom of this
species ranks with those of *N. linearis* and *N. spegazzinii* (the
Acuminatae) in being the smallest in the genus, with an average
chromosome length of 2.2 μ (50, 59). Since *N. palmeri* is mor-
phologically so closely related to *N. trigonophylla* as doubtfully to
deserve specific recognition, the karyotype of the latter is assumed
to be that of the former.

   b. Meiotic chromosome behavior in F_1 interspecific hybrids.
Complete pairing occurs at MI in F_1 *N. trigonophylla* × *N. palmeri,*
whereas in the hybrids of both of these species with *N. debneyi* of
the Suaveolentes there is lack of pairing. Other intersectional
hybrids studied cytologically are F_1 *N. trigonophylla* × *N. pal-
meri* × *N. nudicaulis,* a 24-paired species of the Acuminatae, and F_1
N. palmeri × *N. repanda,* a 24-paired member of the Alatae. In
the former two there is an approximation of "Drosera scheme"
pairing and in the last high variable pairing (4–10) and consid-
erable multivalency. Chromosome behavior at MI in intersub-
generic hybrids involving members of subgenus Rustica (*cf.* p. 551)
and subgenus Tabacum (*cf.* pp. 555, 556) has already been dis-
cussed.

   Section Alatae. a. Karyotypes. Both chromosome number
and chromosome morphology divide this section into a 9-paired, a
10-paired, a 12-paired and a 24-paired group of species (59).
In the 9-paired group *N. sanderae* Hort., although not included
among valid members of the subgenus Petunioides, must be re-
ferred to because this designation has been used in the parentage
of numerous hybrids. Actually, it deserves to rank only as a

horticultural variety of *N. alata,* and the karyotypes of the two
are identical. Otherwise the members of the 9-paired group differ
more or less distinctly in chromosome morphology, although they
give evidence of having been derived from a common karyotype
(3, 50, 59). Thus it is possible in *N. alata, N. bonariensis* and
N. langsdorffii to homologize individual chromosomes on the basis
of size and other morphological distinctions, but the position of
the centromere in corresponding chromosomes may vary from
species to species to affect the karyotype pattern as a result of
chromosomal alterations in the evolution of the species (3, 50, 59).

For these three species a basic karyotype of $5m$ or $sm + 4^2 st$ was
reported (50, 61, 129): in *N. alata* $2 m$ (large) $+ 3 m$ or sm
(small) $+ 4^2 st$, in *N. bonariensis* and *N. langsdorffii* $1 m$ (large) $+ 1$
sm (large) $+ 3 m$ or sm (small) $+ 4^2 st$. Sarana (131) refers to
N. alata as possessing six morphological types of chromosomes.
Avery (2, 3) gives for *N. alata* and *N. langsdorffii* $2 m$ (large) $+ 3$
m or sm (small) $+ 4^2 st$ of varying length, for *N. bonariensis* $1 m$
(large) $+ 1 sm$ (large) $+ 3 m$ or sm (small) $+ 4^2 st$ of varying length.
Results of recent analyses of pollen divisions (59) differ somewhat
from the above reports on the karyotypes of the three species in
question and indicate for *N. alata* $4 m$ (two large) $+ 2 sm$
(small) $+ 3^2 st;$ for *N. bonariensis* $2 m$ (one large) $+ 3 sm$ (one
large) $+ 4^2 st;$ and for *N. langsdorffii* $2 m$ (one large) $+ 4^1 sm$ (one
large) $+ 3^1 st.$

Avery (3) describes in detail the morphology of the chromo-
somes in these species. To be noted are her references to appar-
ently non-nucleolar constrictions (*cf.* p. 540), also observed by
Sarana (131), near the ends of the arms of the two large median
pairs in *N. alata,* and to the satellited pairs in each species, par-
ticularly the large satellite which characterizes the genom of *N.*
langsdorffii. Analyses of pollen grain mitoses (59) confirm these
features of chromosome morphology. The large proximal satellite
in *N. langsdorffii* is striking, being practically as large as the small-
est chromosome in the complement and approximately one-fourth
the length of the chromosome to which it is attached by a thread
nearly as long as the satellite itself. This pair of satellited chromo-
somes is classified in the most recent karyotype formula as sub-
median, although without the satellite it would be subterminal
and was so considered in the karyotype reports earlier given (3,
50).

In addition to necessitating alteration in previously reported formulae of the 9-paired species, pollen division studies (59) have revealed certain other distinctions in chromosome morphology not observed in some previous investigations made of root tip mitoses. Avery (3) refers to two satellited pairs in *N. alata* as indistinguishable from each other and both provided with "small satellites", while in pollen grain mitoses (59) it is apparent that one satellite is distinctly larger than the other (*cf.,* also, 129). The satellite on the small pair of chromosomes in *N. langsdorffii,* the smallest pair in the 9-paired species, appears most frequently, both in pollen and in root tip mitoses, at the periphery of the plate, but it has not yet been determined whether or not it is distal. *N. langsdorffii* is reported to have a third pair of satellited chromosomes (3) which is said to have "partly lost its nucleolus-producing function, since this pair shows a satellite-like body which is close to the head of the chromosome but seldom separated from it by a distinct thread". In pollen divisions this pair has a similar appearance. At least the fact that six nucleoli have been observed (3) would indicate that this pair in addition to the two satellited pairs is nucleolus-organizing.

Chromosome morphology in *N. longiflora* and closely related *N. plumbaginifolia* has been variously described. Resende (127) interprets two pairs of chromosomes in *N. longiflora* as nucleolus-producing, one having proximal and the other distal satellites. Hollingshead (79) refers to chromosomes of *N. longiflora* as straight without "hook" ("heads") at the proximal end and with none showing satellites, although the two shorter pairs are characterized by more or less distinct distal constrictions. Sarana (131) classifies the chromosomes of *N. longiflora* into five morphological types, all rod-like with small "heads", varying only in size and in the possession by one pair of "additions on the distal end which may be satellites". Avery (3) for this species describes ten pairs of "headed" chromosomes (the "head" in some instances not well marked), with continuous slight variation in length, and for *N. plumbaginifolia* a similar karyotype but of shorter and narrower chromosomes. Pollen division analyses of these two 10-paired species (59) confirm Resende's interpretation (127) of two pairs of nucleolus-organizing chromosomes in *N. longiflora,* one with distal and one with proximal satellite, but contradict Avery's comment (3), based on observation of two nucleoli in

N. plumbaginifolia and two to four in *N. longiflora,* that the former has only one satellited pair in contrast to two such pairs in the latter species. In addition, the karyotypes of the two 10-paired species, 10^2 *st,* are practically indistinguishable in size as well as in morphology. Both species have been observed to have two satellited pairs, one of the shorter pairs in each genom bearing a distinct, relatively large distal satellite and one of the longer pairs a minute proximal satellite (59). As already defined (p. 538), subterminal chromosomes (*st*) are those in which the ratio of the long arm to the short arm is 3:1 or greater. In some instances, particularly where the ratio is greater than 3:1, the short arm may appear as a spherical "head" (*cf.* also "knob chromosomes", 4). In certain chromosomes of *N. longiflora, N. plumbaginifolia* and *N. acaulis* (p. 567) the short arm is so reduced that the position of the centromere is not distinguishable.

Studies of pollen grain mitoses (59) confirm the earlier report of the karotype of *N. sylvestris* as consisting of $3\ m + 5^1\ sm + 4^1\ st$ (64). Webber (149) and Brooks (13) classify one of the submedian pairs as subterminal, and both Webber and Kostoff (98) classify one of the submedian pairs as median. Sarana (131) reports eight types of chromosomes on the basis of length, position of centromere and presence of satellites. In pollen divisions we have recently observed (59) on the satellited submedian pair a characteristic feature referred to by Webber as a "subspherical knob at the end of the short arm", and by Goodspeed and Avery (64) as a "large terminal pycnotic chromomere". In *N. sylvestris* only a slight range in chromosome size occurs. In this species the twelve possible trisomic $(2n + 1)$ types have been obtained (64, 65), and the studies of chromosome morphology just referred to made possible the identification of the chromosomes present in triplicate in a number of these types.

The three 24-paired species of the Alatae possess a similar karyotype pattern of predominantly submedian chromosomes (41, 59). The karyotype of *N. repanda* is $10\ m + 8\ sm + 6^3\ st;$ that of *N. stocktonii* and *N. nesophila,* $10\ m + 11^1\ sm + 3^1\ st$ (59). The genom of *N. nesophila* is somewhat smaller than the genoms of the two other species.

b. Meiotic chromosome behavior in F_1 interspecific hybrids. Within the Alatae ten F_1 hybrids representing all combinations of

the 9- and 10-paired species have been studied cytologically (3, 60, 98). In all of these F_1 hybrids except the two between *N. bonariensis* and the two 10-paired species, complete or "Drosera scheme" pairing obtains. An extremely high degree of multivalency occurs in the three F_1 hybrids between the 9-paired species with from one to three trivalents, quadrivalents or quinquevalents in almost every PMC—the mode including in the case of one hybrid three trivalents; in another, one trivalent and one quadrivalent; in the third, one quinquevalent. This multivalency is interpreted (3, 50) as confirming the karyotype evidence that chromosomal alterations have occurred in the evolution of these species. F_1 *N. bonariensis* × *N. longiflora* and × *N. plumbaginifolia* show almost complete lack of pairing, *i.e.*, zero to 3 pairs (Kostoff, 0–5). In view of the complete or "Drosera scheme" pairing in other intrasectional hybrids involving these species it has been suggested (3) that this absence of significant pairing is due to environmental and other factors and is not, therefore, a reflection of equivalent lack of genic homology.

The 12-paired species, *N. sylvestris*, has been crossed within the section only with 10-paired *N. longiflora* and with 24-paired *N. repanda*. Both F_1 hybrids show lack of pairing. Complete pairing has been found to occur in the three F_1 hybrids between the 24-paired species of the Alatae, *N. repanda*, *N. stocktonii* and *N. nesophila* (41).

Numerous F_1 hybrids between members of the Alatae and species of other sections of subgenus Petunioides have been studied cytologically. Of 14 such hybrids involving members of the Suaveolentes (*cf.* list p. 579) all have been consistently reported to exhibit no pairing (0–4, mode 0). Similar lack of pairing is shown by F_1 *N. bigelovii* × *N. sylvestris* and F_1 *N. undulata* × *N. longiflora* and × *N. langsdorffii*. By contrast Kostoff reports (98) high variable pairing, in certain instances approaching "Drosera scheme" pairing, in four F_1 hybrids: *N. miersii* × *N. langsdorffii*, *N. noctiflora* × *N. alata*, × *N. sanderae*, and × *N. langsdorffii*. Already noted is Kostoff's report of similar pairing in F_1 hybrids between *N. tabacum* (subgenus Tabacum) and three of the 9- and 10-paired members of the Alatae (*cf.* p. 557), and between three species of subgenus Rustica (*N. glauca*, *N. paniculata* and *N. rustica*) and various of the 9- and 10-paired members of the Alatae (*cf.* pp. 549,

550, 551). Evidence contradicting Kostoff's results has been reported in the case of two hybrids, F₁ *N. paniculata × N. langsdorffii* (*cf.* p. 549) and F₁ *N. glauca × N. plumbaginifolia* (*cf.* p. 550).

Reference has also been made to the high variable pairing occurring in F₁ *N. repanda × N. palmeri* (*cf.* p. 562) and to the significance of meiotic chromosome behavior in F₁ hybrids between *N. sylvestris* and all members of subgenus Tabacum for interpretation of the amphidiploid origin of *N. tabacum* (*cf.* p. 557).

Section Noctiflorae. a. Karyotypes. The three 12-paired species that constitute this section (*N. noctiflora, N. acaulis, N. petunioides*) are practically equivalent in karyotype pattern (59). With the exception of one submedian pair in *N. petunioides*, only subterminal chromosomes occur. In *N. acaulis* and in *N. petunioides* satellites were seen on two pairs of subterminal chromosomes, one of them on the long arm (distal) in *N. acaulis* (59). *N. noctiflora*, however, appears to have three satellited pairs of chromosomes, two with proximal and one with distal satellites (50, 59). In *N. acaulis* the chromosomes are considerably larger than in the two other species—indeed, in size they are outstanding in the genus —and the position of the centromere is usually so nearly terminal in the majority of the chromosomes that, as in *N. longiflora* (*cf.* p. 565), the short arms do not appear even as "heads".

b. Meiotic chromosome behavior in F₁ interspecific hybrids. No information is available for hybrids involving *N. acaulis*, but F₁ *N. noctiflora × N. petunioides* exhibits complete pairing at MI. Of intersectional hybrids F₁ *N. corymbosa* (Acuminatae) *× N. petunioides* shows high variable pairing (3–10, mode 7), and Kostoff (98) reports "Drosera scheme" pairing or an approximation thereof for F₁ *N. noctiflora × N. alata, × N. sanderae* and *× N. langsdorffii* (Alatae). By contrast is the almost complete lack of pairing that obtains in intersubgeneric hybrids of *N. noctiflora* with *N. paniculata*, with *N. rustica* and with *N. glauca* of subgenus Rustica (*cf.* 549, 550, 551).

Section Acuminatae. a. Karyotypes. This section consists of seven 12-paired species and three 24-paired species. Chromosome size and morphology divide the 12-paired species into two groups: for *N. acuminata, N. pauciflora, N. attenuata, N. corymbosa* and *N. miersii* the karyotype is 2 *m* + 6¹ *sm* + 4¹ *st*; for *N. spegazzinii* and *N. linearis*, with much smaller chromosomes, it is 1 *m* + 3¹

$sm + 8^1 st$ (59). In contrast to the uniformly small chromosomes in the species of the latter group, in the former, and more particularly in *N. acuminata* and *N. attenuata,* there are marked size differences within the genoms, the two median and two of the submedian chromosomes being considerably longer than the other submedian and subterminal ones. Additional constrictions (*cf.* p. 540) are present near the distal end of one of the longer submedian pairs of chromosomes in some of the species of this group. In the chromosomes of the Acuminatae the position of the centromere is often unusually conspicuous with the result that the "heads" which represent the exceedingly short arms of the subterminal chromosomes are clearly distinguishable (*cf. N. longiflora,* p. 565).

Of the three 24-paired species, *N. bigelovii* and *N. clevelandii* are similar in karyotype ($8 m + 10^1 sm + 6^1 st$) but differ somewhat in the smaller average size of the chromosomes of the latter species (59). Within these genoms, as in the case of most of the 12-paired species of this section, there are marked differences in chromosome size, two median and two submedian pairs being among the longer chromosomes of the complements. Here, too, additional apparently non-nucleolar constrictions occur near the distal ends of one of the longer submedian pairs, and the short arms of some of the subterminal pairs appear as conspicuous "heads". In *N. bigelovii,* though not as yet in *N. clevelandii,* two satellited pairs (one submedian and one subterminal) have been observed. The karyotype of the third 24-paired species of the Acuminatae, *N. nudicaulis,* ($5 m + 12 sm + 7^1 st$) consists of chromosomes even smaller than those of *N. clevelandii,* but lacks the size differences that mark the genom of that species. Only one satellited pair has been identified (59).

b. Meiotic chromosome behavior in F₁ interspecific hybrids. Of the 12-paired species, each (with the exception of *N. spegazzinii*) has been crossed with at least one other, *N. acuminata* and *N. corymbosa* with two others. In F₁ *N. corymbosa × N. acuminata* and F₁ *N. pauciflora × N. acuminata* complete pairing occurs; in F₁ *N. corymbosa × N. linearis* and F₁ *N. attenuata × N. miersii* the author finds high variable to complete pairing, five to 11 pairs with mode at 9, and three to 12 pairs with mode at 9, respectively. In the last named hybrid Kostoff reports (98) only one to seven pairs. The three F₁ hybrids between the 24-paired species have been

studied. In F₁ *N. bigelovii* × *N. clevelandii* practically complete pairing occurs. The author's analyses of F₁ *N. bigelovii* × *N. nudi-caulis* show a pairing range of 2 to 12 with mode at seven pairs (60), while others have reported no pairing (16), and zero to 3, rarely 4 (98). In F₁ *N. clevelandii* × *N. nudicaulis* the pairing ranges from zero to nine pairs (mode at 6), with one or two trivalents occurring in approximately 25% of the PMC. Four F₁ hybrids between the 24-paired species and the 12-paired species of the Acu-minatae (*N. bigelovii* and *N. clevelandii* with both *N. attenuata* and *N. miersii*) all show "Drosera scheme" pairing, or a close ap-proximation of it, with some multivalency. This multivalency is very extensive in the case of F₁ *N. bigelovii* × *N. attenuata,* 50% of the PMC analyzed showing one or more trivalents, or frequently quadrivalents, and occasionally even higher valencies (50, 60).

Already noted is the occurrence of no pairing in F₁ *N. un-dulata* × *N. attenuata* (*cf.* p. 562), of high variable pairing in F₁ *N. corymbosa* × *N. petunioides* (*cf.* p. 567) and in F₁ *N. miersii* × *N. langsdorffii* (*cf.* p. 566), and of "Drosera scheme" pairing in F₁ *N. nudicaulis* × *N. palmeri* and × *N. trigonophylla* (*cf.* p. 562). Other intersectional hybrids studied are ten made between members of the Acuminatae and species of the Suaveolentes. All of these F₁ hybrids (*cf.* list p. 579), several of them studied by both Goodspeed (50, 60) and Kostoff (98) and a few by other investigators also, consistently show lack of pairing.

Lack of pairing in F₁ hybrids between three 12-paired species of the Acuminatae and *N. rustica* (*cf.* p. 551), between one of them and *N. paniculata* (*cf.* p. 549), and between two of them and *N. tabacum* (*cf.* p. 556), has already been referred to. Similar lack of pairing has been noted in F₁ hybrids of *N. bigelovii* with *N. syl-vestris,* with four species of subgenus Rustica (*cf.* pp. 549, 551), and with three species of subgenus Tabacum (*cf.* p. 555) ; and of *N. nudicaulis* with four species of subgenus Tabacum (*cf.* pp. 555, 556), and with two species of subgenus Rustica (*cf.* pp. 549, 551). A somewhat higher degree of pairing occurs in F₁ *N. bigelovii* × *N. tabacum,* with a range of zero to 10 and mode at 4 pairs.

Section Suaveolentes. a. Karyotypes. Of the 15 species of this section the chromosome numbers and morphology of 13 have been investigated. These 13 species show a chromosome number range of from 16 to 24 pairs and many similarities in their karyo-

types. The various complements are composed of median, sub-
median and subterminal chromosomes in ratios varying from
species to species. There are four 16-paired species (*N. suaveo-
lens, N. maritima, N. velutina, N. exigua*), one 18-paired (*N.
gossei*), two 19-paired (*N. excelsior, N. benthamiana*), two 20-
paired (*N. megalosiphon, N. goodspeedii*), one 21-paired (*N. occi-
dentalis*), one 22-paired (*N. rotundifolia*), and two 24-paired (*N.
debneyi, N. fragrans*). A 32-paired member of the Suaveolentes,
referred to by Kostoff as "*N. eastii*" (95), is not here recognized
as a species, since it has not been formally described and for other
reasons referred to below. Chromosome numbers for 11 of these
13 species have already been reported (48, 50, 98), and the remain-
ing two have been determined by Wheeler (154). She also has
determined as 19-paired both *N. benthamiana* and *N. excelsior*
which were cited by Kostoff as 18-paired (98). Descriptions of
the karyotype pattern of five species have been given (50, 152)
and are confirmed by more recent observations made on pollen
grain mitoses (154).

Although there is a considerable size range (*ca.* 2–5 μ in length)
and the extremes may occur within a single species, close inter-
gradation in size even in the species of greatest range occurs. The
genom of every species of the Suaveolentes possesses subterminal
chromosomes, as do the genoms of all species (except *N. undulata*)
of the five other sections of subgenus Petunioides already reviewed.
Apparently the relative number of subterminal chromosomes in-
creases with increase of chromosome number, and correspondingly,
the average chromosome length decreases.

Although distinctions in chromosome morphology are difficult to
make in some instances, Wheeler's studies (154) of karyotypes in
the 13 species of the Suaveolentes which she has investigated cyto-
logically may be interpreted as follows. The four 16-paired species
show $8^1 m + 4^1 sm + 4 st$ (*N. suaveolens* and *N. maritima*), $8^1 m + 4$
$sm + 4^1 st$ (*N. velutina*) or $6^1 m + 5 sm + 5^1 st$ (*N. exigua*); the
18-paired species (*N. gossei*) $5 m + 4^1 sm + 9^1 st$. Of the two 19-
paired species *N. excelsior* shows $5 m + 4^1 sm + 10^1 st$ and *N. ben-
thamiana* $1 m + 4 sm + 14^1 st$; of the two 20-paired species *N.
megalosiphon* shows $2 m + 4^1 sm + 14^1 st$ and *N. goodspeedii* 6^1
$m + 4^1 sm + 10 st$. The 21- and 22-paired species (*N. occidentalis*
and *N. rotundifolia*) have much the same karyotype, $4 m + 3$

$sm + 14^1 st$ and $4^1 m + 3 sm + 15^1 st$, respectively, while the two 24-paired species ($N. debneyi$ and $N. fragrans$) consist of $4^1 m + 1 sm + 19^1 st$ and $2^1 m + 6 sm + 16^1 st$, respectively. Thus the karyotypes of $N. suaveolens$ and $N. maritima$, $N. benthamiana$, $N. megalosiphon$ and $N. debneyi$ are most extreme in that they are composed predominantly of chromosomes of one morphological type or the other. The occurrence of one pair of satellited subterminal chromosomes has in the above formulae been noted for every species except $N. suaveolens$, $N. maritima$ and $N. goodspeedii$. In these three species the chromosome pair involved has been classified as submedian by Wheeler, although it appears to be on the borderline between submedian and subterminal. Apparently there is some evidence (154) that a second subterminal pair is satellited in $N. debneyi$ and in $N. fragrans$. In one to several median or submedian pairs in every species except $N. benthamiana$ Wheeler notes an additional constriction ($cf.$ p. 540), and in $N. benthamiana$ she has observed one submedian pair possessing a large chromosomal segment, simulating the large satellite in $N. langsdorffii$ ($cf.$ p. 563), separated by a distinct thread from the body of the chromosome.

 b. *Meiotic chromosome behavior in F_1 interspecific hybrids.* The extent and character of pairing at MI has been studied in a total of 63 F_1 hybrids involving 12 of the species of this section, the karyotypes of which were described above. Thirty of these hybrids are intrasectional, 26 are intersectional and 7 involve species of the two other subgenera. Reference to the appended list of F_1 interspecific hybrids (p. 579) will permit identification of those the meiotic behavior of which will be briefly referred to below. The evidence in this connection is derived from the work of Wheeler upon 26 intrasectional hybrids (154), that of Goodspeed (50, 60) upon nine intersectional and intersubgeneric hybrids, and that of Kostoff (98) upon 13 of the same hybrids and upon 28 additional ones. In four instances (16, 44) others have provided information on meiotic chromosome behavior in F_1 hybrids investigated by Wheeler, Goodspeed or Kostoff. In general, the results of the various investigators confirm one another, except that Kostoff in certain cases reports a somewhat higher degree of pairing in intrasectional hybrids than is found by Wheeler.

 Of the six hybrids which have been studied between the four 16-paired species, all show complete or almost complete pairing.

In F$_1$ *N. maritima* × *N. suaveolens* 16 pairs occur, and for F$_1$ *N. exigua* × *N. suaveolens* and F$_1$ *N. exigua* × *N. maritima* up to 16 pairs have been reported. Of the remaining three hybrids—*N. velutina* with *N. maritima*, *N. exigua* and *N. suaveolens*—the first two, according to Wheeler's evidence, exhibit 12 to 15 pairs and the last 13 to 15 pairs, while Kostoff reports for these three hybrids 13 to 16, 15 to 16 and 14 to 16 pairs, respectively. The four hybrids between 16- and 18-paired species show "Drosera scheme" pairing or an approximation thereof. The same is true of three of the six F$_1$ hybrids between 16- and 20-paired species, of one of the two between 18- and 19-paired species, and of a single hybrid in each case between 16- and 22-, 16- and 24-, 20- and 22-, and 20- and 24-paired species. Among these Wheeler notes the highest degree of pairing in the following three F$_1$ hybrids: *N. suaveolens* × *N. maritima*, *N. goodspeedii* × *N. suaveolens* and *N. maritima* × *N. debneyi*. An amount of pairing somewhat less complete than in the foregoing hybrids but still in the high variable category obtains for the 11 remaining F$_1$ intrasectional hybrids investigated. Of these, four involving *N. benthamiana* with *N. suaveolens*, *N. gossei*, *N. megalosiphon* and *N. debneyi* show the lowest amount of pairing among all the intrasectional hybrids of the Suaveolentes, with a combined range of 6 to 17 and a mode at 10 or 11 pairs. Five other hybrids in this category involve *N. megalosiphon* with *N. suaveolens*, *N. exigua*, *N. velutina*, *N. gossei* and *N. debneyi*, and the remaining two hybrids showing high variable pairing are F$_1$ *N. occidentalis* × *N. gossei* and that between the two 20-paired species, F$_1$ *N. goodspeedii* × *N. megalosiphon*, where the pairing ranges from 12 to 16 pairs. Wheeler finds a high degree of multivalent formation in most of the 26 intrasectional hybrids she has studied. Indeed, of 30 such hybrids, in all but four one or more trivalents were present in a high percentage of the PMC. In one hybrid between two 16-paired species up to five trivalents occurred. A quadrivalent has occasionally been seen in certain of the hybrids.

By contrast with the high degree of homology exhibited by the genoms of the parents of all the F$_1$ hybrids made between the species of section Suaveolentes, is the almost complete lack of pairing consistently shown in intersectional and intersubgeneric F$_1$ hybrids involving species of that section. As found principally by

Kostoff (98), but also by Goodspeed (50, 60), a pairing range of zero to 2 or 3 is characteristic of 22 such hybrids, a range of zero to 4 or 5 of ten, and in one case a range of zero to 6. These results refer to nine hybrids between species of section Alatae (*N. alata, N. sanderae, N. langsdorffii, N. longiflora, N. plumbaginifolia,* and *N. repanda*) and 16-paired species, three of the same species (*N. alata, N. sanderae,* and *N. longiflora*) and an 18-paired species, and two (*N. longiflora* and *N. plumbaginifolia*) and a 20-paired species. Similarly, ten F_1 hybrids involve 16-, 19-, 20- and 24-paired species and *N. attenuata, N. miersii, N. nudicaulis, N. bigelovii* and *N. clevelandii,* members of the Acuminatae. Hybrids between a 24-paired species and *N. trigonophylla* and *N. palmeri* of section Trigonophyllae have also been studied. Intersubgeneric hybrids, all showing lack of pairing, include four involving a member of subgenus Rustica (F_1 *N. suaveolens* × *N. solanifolia,* × *N. glauca* and × *N. rustica,* and F_1 *N. glauca* × *N. megalosiphon*) and three in which a species of subgenus Tabacum is concerned (F_1 *N. suaveolens* × *N. tabacum* and × *N. glutinosa,* and F_1 *N. debneyi* × *N. tabacum*).

An additional series of F_1 hybrids of which a 32-paired member of the Suaveolentes is a parent has been reported principally by Kostoff. He has applied the designation "*N. eastii*" to this 32-paired race, since he considers that he has demonstrated its origin as a probable amphidiploid of F_1 *N. suaveolens* × *N. maritima.* Wheeler (153), on the other hand, refers to a 32-paired race which is probably equivalent, as a tetraploid of *N. suaveolens.* Hybrids between "*N. eastii*" and *N. alata, N. sanderae, N. longiflora, N. plumbaginifolia* (98) and *N. bigelovii* (60) show 16 pairs. Since in F_1 *N. suaveolens* × *N. maritima* complete pairing occurs, such consistent evidence of autosyndesis within the gamete set of "*N. eastii*" might be considered indicative of its origin through either allo- or autopolyploidy. In F_1 hybrids of "*N. eastii*" with the 16-paired species *N. suaveolens* and *N. maritima* Kostoff finds 14 to 16 bivalents and zero to three trivalents, and for F_1 "*N. eastii*" × *N. suaveolens* Wheeler reports up to 15 trivalents. Kostoff refers to pairing in F_1 "*N. eastii*" × *N. velutina,* × *N. gossei* and × *N. megalosiphon* as variable with some trivalency. For convenience of reference F_1 hybrids involving this 32-paired race of section Suaveolentes are included in the subjoined list of interspecific hybrids under "*N. eastii*".

Comment. For subgenus Petunioides cytological information in general confirms and amplifies the morphological and distributional evidence which contributed largely to the formulation of the sectional arrangement. Thus, almost identical karyotypes of uniformly subterminal chromosomes characterize the three species which have been studied in the 12-paired section Noctiflorae, and complete pairing occurs in an F_1 hybrid between them. Similarly, chromosome conjugation is complete in the F_1 hybrid between the two species of the Trigonophyllae. The close relationship of the seven 12-paired species of the Acuminatae is clearly shown by their possession of a similar karyotype pattern and by the extensive pairing in F_1 hybrids involving them, and equivalent cytological evidence relates *N. bigelovii* and *N. clevelandii,* two of the three 24-paired species of that section. Finally, and in particular, similarities in karyotype patterns and extensive pairing in F_1 intrasectional hybrids confirm the evidence from external morphology which indicates the close relationship of the species of the Suaveolentes. The species of the remaining two sections (Undulatae and Alatae) show in some instances an equivalent and in others a less close integration on the basis of the cytological as well as the morphological and distributional information available.

In the Undulatae the 12-paired species, *N. undulata* and *N. wigandioides,* exhibit a certain morphological resemblance to members of subgenus Rustica and subgenus Tabacum, respectively. Occurrence of "Drosera scheme" pairing in F_1 *N. rustica* × *N. undulata* and similarity of the karyotypes of *N. undulata* and members of subgenus Rustica, together with the extent of pairing in F_1 *N. wigandioides* × *N. glutinosa* (Tomentosae) contribute to the impression that the Undulatae may represent a transitional section somewhat uniting subgenus Petunioides with the two other subgenera. The remaining member of the Undulatae is 24-paired *N. arentsii.* To the parentage of this amphidiploid species progenitors of the two other species of the Undulatae entered in as shown, first, by "Drosera scheme" relations on the two sides of the triangle uniting as hybrids *N. arentsii* with *N. undulata* and *N. wigandioides* and, second, by the almost complete reflection of the karyotypes of the latter two species in that of the former.

Morphologically, section Alatae contains a nucleus of five 9- or 10-paired species, the relationships of certain of them being

obviously close.   In terms of karyotype patterns there are essential
identities and also dissimilarities among these five species, whereas
the high degree of pairing and multivalency in F_1 hybrids between
them indicates their fundamental relationships as well as the occur-
rence of quantitative chromosomal alterations during their evolu-
tion.   In addition to these nucleus species there are in the Alatae
the three 24-paired species, *N. repanda, N. stocktonii* and *N. ne-
sophila*.   Similar karyotypes and complete pairing in F_1 hybrids
between them indicate, as does the morphological and distributional
evidence, the closeness of their relationship. In floral and in
chromosomal morphology they are somewhat allied to the 9- and
10-paired species.   In view of this fact and because of the approxi-
mation of "Drosera scheme" pairing in F_1 *N. repanda × N. palmeri*
it is suggested that the 24-paired species of the Alatae represent
amphidiploids, progenitors of a 12-paired ancestor of the 9- and
10-paired members of the Alatae and of members of the Tri-
gonophyllae having entered into their origin.   The remaining
species, 12-paired *N. sylvestris,* although somewhat outlying in
many respects, is, however, included in the Alatae because of mor-
phological resemblances to other members of this section. Its
karyotype is not unrelated to the basic karyotype of the 9-paired
species.   Only in the case of F_1 *N. tabacum × N. sylvestris,* where
"Drosera scheme" relations obtain, is there significant pairing in
the relatively few F_1 hybrids involving *N. sylvestris* which it has
been possible to obtain.

   Cytological evidence indicates in some instances considerable
and in others little relationship between sections of subgenus
Petunioides.   Thus, high variable pairing in an F_1 hybrid between
N. petunioides (Noctiflorae) and *N. corymbosa* (Acuminatae)
points to genic similarities between members of different sections
which also find expression in the possession by the two species of
certain morphological characters in common.   The occurrence of
amphidiploid species and the presence in different sections of the
modern descendants of their putative ancestors also serve to relate
certain subdivisions of this subgenus.   The evidence in this con-
nection concerning the amphidiploid origin of these 24-paired
species of the Alatae has been noted above.   In the amphidiploid
origin of the two closely related 24-paired species of the Acuminatae
(*N. bigelovii* and *N. clevelandii*) a progenitor of the 12-paired

members of the same section is involved, as evidenced by "Drosera scheme" pairing exhibited by their F_1 hybrids with such 12-paired species. Equally significant cytological information pointing to present day species, an ancestor of which represented the other parent of these two amphidiploid species of the Acuminatae, is not available. Possibly modern descendants of such a parental species are no longer in existence. It is to be noted, however, that we find as many as ten bivalents in F_1 *N. bigelovii × N. tabacum,* and since *N. sylvestris,* a member of the Alatae, is represented in the amphidiploid origin of *N. tabacum* it may be that a 12-paired progenitor of the Alatae entered into the origin of *N. bigelovii* and *N. clevelandii.* The third 24-paired member of the Acuminatae, *N. nudicaulis,* apparently involved in its amphidiploid origin a progenitor of *N. trigonophylla* (Trigonophyllae), since the karyotypes of the two species are similar and their F_1 hybrid shows "Drosera scheme" pairing. Our observation of up to 12 bivalents in F_1 *N. nudicaulis × N. bigelovii* and up to nine in F_1 *N. nudicaulis × N. clevelandii,* together with morphological characters which *N. nudicaulis* has in common with other species of the Acuminatae, suggests that a progenitor of some member of the Acuminatae represents the other parental species concerned in the amphidiploid origin of *N. nudicaulis.*

By contrast with such direct and indirect indications of relationship between sections of subgenus Petunioides is the cytological indication of a genic isolation (lack of pairing in intersectional and intersubgeneric F_1 hybrids) of the Suaveolentes equivalent to its geographic isolation. However, there is, according to Wheeler (154), some evidence that the Suaveolentes may have been derived from the source from which the 9- and 10-paired species of the Alatae, the 12-paired Noctiflorae and the 12-paired Acuminatae have differentiated.

The aneuploid series—16, 18, 19, 20, 21, 22, 24—into which fall the chromosome numbers of the 13 species investigated cytologically points to a fundamental hybrid structure for the Suaveolentes, and the increase in number of subterminal chromosomes with increasing chromosome number might be reconciled with this concept. If the hybrids involving "*N. eastii*" are included, meiotic chromosome behavior is known in a total of 73 F_1 interspecific hybrids of which members of the Suaveolentes are parents. As

already noted all these hybrids except those in which "*N. eastii*" is concerned exhibit four of the five types of pairing defined in the résumé of the cytology of the genus (*cf.* p. 542). Thus in the majority of the intrasectional hybrids either complete or "Drosera scheme" pairing or approximations thereof occur, with the remaining hybrids showing high variable pairing, while all intersectional and intersubgeneric hybrids show lack of pairing. If "*N. eastii*" is to be considered a species, a sixth category of meiotic behavior at MI in interspecific hybrids of *Nicotiana* must be added, since in five intersectional and intersubgeneric hybrids involving "*N. eastii*" autosyndesis occurs.

SUMMARY

Current morphological and distributional information indicates that the genus *Nicotiana* consists of somewhat less than 60 valid species, the natural distribution of which is confined to temperate South America, western North America, Australia and a few South Pacific islands. From the beginning, efforts to organize the genus taxonomically resulted in the recognition of at least three major subdivisions, relationships within each of which were more or less clearly expressed morphologically. Less than half the total number of species now known were considered in these older taxonomic organizations. Addition of numerous new species, increased knowledge of the morphology, range of distribution and variation of the classical species, and, in particular, information concerning chromosome number and morphology and chromosome behavior in F_1 interspecific hybrids have resulted in revaluations of previous taxonomic concepts.

Efforts to correlate accumulating morphological, distributional and cytological evidence revealed the presence of a series of genetic centers or groupings of species within the genus. The members of these "genetic groups" were considered to possess distinctive morphological, distributional and cytological characters sufficiently in common, on the one hand, to demonstrate that phylogenetic relationships within the individual groups were relatively intimate, and, on the other, to set apart the groups themselves one from another. Somewhat later most of these "genetic groups" were referred to as sections in the taxonomic sense. In a recent taxonomic revision of the genus three of the major subdivisions of the

classical taxonomic organizations were retained as subgenera
(Rustica, Tabacum, Petunioides) under which a total of 11 sections
were described.

Available cytological information concerning the genus consists
of determination by a number of investigators of chromosome mor-
phology in 55 of the 58 recognized species of *Nicotiana* and of the
amount and quality of pairing in some 210 F$_1$ interspecific hybrids
involving as parents 53 species. This information can be organ-
ized as follows to reflect the extent to which the most recent taxo-
nomic arrangement is expressive of what appear to be fundamental
phylogenetic relationships.

In terms of chromosome morphology it has been shown that
there is no common karyotype pattern for *Nicotiana*, but in the
genus as a whole median and submedian predominate over sub-
terminal chromosomes in the ratio of 5:3. Certain other cyto-
logical features are characteristic: (*a*) the possession by consid-
erably over one-half the species of genoms containing 12 or 24
pairs of chromosomes; (*b*) the relatively small to medium size
of the chromosomes; (*c*) the presence in every genom of at least
one pair of satellited chromosomes, usually a subterminal pair with
proximal satellites. In addition, distinctions in chromosome mor-
phology characterize individual species, sections and even sub-
genera, as follows: (*a*) a characteristic ratio of median and sub-
median to subterminal chromosomes serves to distinguish the three
subgenera from one another—Rustica 9:1, Tabacum 5:2, Petuni-
oides 4:3; (*b*) in subgenus Rustica, which contains three sections,
the karyotype formulae are practically identical for all but one of
the nine species; in the two other subgenera there is no comparable
uniformity in karyotype, although, as just noted, their basic karyo-
type patterns set them apart from subgenus Rustica and from each
other; (*c*) in section Tomentosae of subgenus Tabacum, three of
the five species closely related morphologically possess practically
identical karyotypes, the remaining section consisting of a single
amphidiploid species; (*d*) each of four of the six sections of sub-
genus Petunioides possesses a particular karyotype characteristic in
each case of the nucleus species; thus these four sections are set
apart from one another in terms of chromosome morphology; (*e*)
the karyotypes of the nine amphidiploid species reflect in more or
less detail the karyotypes of the modern relatives of their postu-
lated ancestors.

From the point of view that chromosome homology reflected in the formation of bivalents (or higher valencies) at MI is indicative of the extent to which genes and their arrangement in the conjugating chromosomes are common or similar, the amount of pairing in F_1 interspecific hybrids has been employed to provide evidence concerning the relationships of the species in each case involved: (a) approximately 90% of the F_1 intrasectional hybrids indicate close relationship of the species concerned by exhibiting complete or almost complete pairing of their genoms;* (b) by contrast, in only 10% of the hybrids between species of different sections of a given subgenus does appreciable pairing occur; (c) corresponding to the more distant relationship of the species concerned which is postulated in the taxonomic arrangement, none of the F_1 hybrids which involve species of different subgenera shows significant pairing, except certain of those in which N. glauca or a species of the Alatae is a parent; (d) in all intra- and inter-subgeneric F_1 hybrids between any of the nine amphidiploid species and the descendants of their putative progenitors, "Drosera scheme" pairing or an approximation thereof occurs; (e) on the other hand, when these same amphidiploid species are crossed with species other than those related to their parentage, 85% of the F_1 hybrids exhibit little or no pairing.

Thus, the evidence of phylogenetic relationships in *Nicotiana* which is intrinsic in the morphological and distributional data appears to be sufficiently confirmed and amplified by the cytological conclusions to justify the current taxonomic arrangement of the genus.

LIST OF F_1 INTERSPECIFIC HYBRIDS OF NICOTIANA INVESTIGATED CYTOLOGICALLY

Hybrids are listed alphabetically; reciprocals not listed. Page references are to the present article; others are to the bibliography. Except in a few instances the results of Kostoff and of Goodspeed are referred to only in articles containing résumés of cytological behavior.

N. alata × N. bonariensis—p. 566 (3, 60, 97, 98)
N. alata × N. langsdorffii—p. 566 (3, 16, 60, 83, 98)

* Hybrids involving amphidiploid species are obviously not included in points (a), (b) and (c).

N. arentsii × *N. wigandioides*—p. 561 (57, 60)
N. attenuata × *N. glauca*—p. 549 (60)
N. attenuata × *N. miersii*—p. 568 (60, 98)
N. benavidesii × *N. glutinosa*—pp. 549, 555 (60)
N. benavidesii × *N. raimondii*—p. 548 (60)
N. benavidesii × *N. solanifolia*—p. 548 (60)
N. benthamiana × *N. debneyi*—p. 572 (154)
N. benthamiana × *N. megalosiphon*—p. 572 (154)
N. benthamiana × *N. suaveolens*—p. 572 (154)
N. bigelovii × *N. attenuata*—p. 569 (50, 60, 97, 98)
N. bigelovii × *N. benthamiana*—pp. 569, 573 (98)
N. bigelovii × *N. clevelandii*—p. 569 (60)
N. bigelovii × *N. glauca*—pp. 549, 569 (50, 60, 97, 98)
N. bigelovii × *N. glutinosa*—pp. 555, 569 (17, 60, 68, 97, 98)
N. bigelovii × *N. megalosiphon*—pp. 569, 573 (97, 98)
N. bigelovii × *N. nudicaulis*—p. 569 (16, 60, 97, 98)
N. bigelovii × *N. paniculata*—pp. 549, 569 (97, 98)
N. bigelovii × *N. solanifolia*—pp. 549, 569 (50, 60)
N. bigelovii × *N. suaveolens*—pp. 569, 573 (50, 60, 68, 92, 97, 98)
N. bigelovii × *N. sylvestris*—pp. 566, 569 (50, 60, 98)
N. bigelovii × *N. tabacum*—pp. 556, 569 (17, 60, 97, 98)
N. bigelovii × *N. tomentosa*—pp. 555, 569 (97, 98)
N. bigelovii × *N. tomentosiformis*—pp. 555, 569 (97, 98)
N. bonariensis × *N. plumbaginifolia*—pp. 566 (98)
N. clevelandii × *N. attenuata*—p. 569 (60, 98)
N. clevelandii × *N. exigua*—pp. 569, 573 (98)
N. clevelandii × *N. nudicaulis*—p. 569 (60)
N. corymbosa × *N. acuminata*—p. 568 (60)
N. corymbosa × *N. linearis*—p. 568 (60)
N. corymbosa × *N. petunioides*—pp. 567, 569 (60)
N. debneyi × *N. nudicaulis*—pp. 569, 573 (50, 60, 97, 98)
N. debneyi × *N. palmeri*—pp. 562, 573 (98)
N. debneyi × *N. suaveolens*—p. 572 (98)
N. debneyi × *N. tabacum*—pp. 556, 573 (98)
N. debneyi × *N. trigonophylla*—pp. 562, 573 (60, 97, 98)
"*N. eastii*" × *N. alata*—p. 573 (97, 98)
"*N. eastii*" × *N. bigelovii*—p. 573 (60)
"*N. eastii*" × *N. longiflora*—p. 573 (97, 98)
"*N. eastii*" × *N. gossei*—p. 573 (98)

"*N. eastii*" × *N. maritima*—p. 573 (97, 98)
"*N. eastii*" × *N. megalosiphon*—p. 573 (98)
"*N. eastii*" × *N. plumbaginifolia*—p. 573 (98)
"*N. eastii*" × *N. sanderae*—p. 573 (97, 98)
"*N. eastii*" × *N. suaveolens*—p. 573 (97, 98, 152)
"*N. eastii*' × *N. velutina*—p. 573 (98)
N. exigua × *N. maritima*—p. 572 (97, 98)
N. exigua × *N. megalosiphon*—p. 572 (98, 154)
N. exigua × *N. nudicaulis*—pp. 569, 573 (98)
N. exigua × *N. repanda*—pp. 566, 573 (98)
N. exigua × *N. suaveolens*—p. 572 (97, 98)
N. glauca × *N. alata*—pp. 550, 566 (83, 97, 98)
N. glauca × *N. benavidesii*—p. 548 (60)
N. glauca × *N. langsdorffii*—pp. 550, 566 (83, 97, 98)
N. glauca × *N. longiflora*—pp. 550, 566 (83, 97, 98)
N. glauca × *N. megalosiphon*—pp. 550, 573 (98)
N. glauca × *N. noctiflora*—pp. 549, 567 (50, 60, 97, 98)
N. glauca × *N. plumbaginifolia*—pp. 550, 566 (97, 98, 125)
N. glauca × *N. sanderae*—pp. 550, 566 (83, 97, 98)
N. glauca × *N. tomentosa*—pp. 550, 555 (60, 83, 97, 98)
N. glauca × *N. tomentosiformis*—pp. 550, 555 (83, 97, 98)
N. glutinosa × *N. glauca*—pp. 550, 555 (83, 97, 98)
N. glutinosa × *N. otophora*—p. 554 (60)
N. glutinosa × *N. sylvestris*—pp. 555, 567 (50, 60, 97, 98)
N. glutinosa × *N. tomentosa*—pp. 554 (50, 60, 97, 98)
N. glutinosa × *N. tomentosiformis*—p. 554 (60, 97, 98, 99)
N. glutinosa × *N. wigandioides*—pp. 555, 562 (39, 50, 60, 97, 98)
N. goodspeedii × *N. debneyi*—p. 572 (154)
N. goodspeedii × *N. exigua*—p. 572 (154)
N. goodspeedii × *N. megalosiphon*—p. 572 (154)
N. goodspeedii × *N. rotundifolia*—p. 572 (154)
N. goodspeedii × *N. suaveolens*—p. 572 (154)
N. gossei × *N. alata*—pp. 566, 573 (97, 98)
N. gossei × *N. benthamiana*—p. 572 (154)
N. gossei × *N. excelsior*—p. 572 (154)
N. gossei × *N. exigua*—p. 572 (154)
N. gossei × *N. longiflora*—pp. 566, 573 (97, 98)
N. gossei × *N. sanderae*—pp. 566, 573 (97, 98)
N. gossei × *N. suaveolens*—p. 572 (98, 154)

N. knightiana × *N. cordifolia*—p. 548 (60)
N. langsdorffii × *N. bonariensis*—p. 566 (3, 60, 97, 98, 117)
N. langsdorffii × *N. sanderae*—p. 566 (83, 97, 98)
N. longiflora × *N. alata*—p. 566 (3, 60, 98)
N. longiflora × *N. bonariensis*—p. 566 (3, 60, 98)
N. longiflora × *N. langsdorffii*—p. 566 (3, 60, 97, 98)
N. longiflora × *N. plumbaginifolia*—p. 566 (3, 60, 97, 98)
N. longiflora × *N. sylvestris*—p. 566 (98)
N. maritima × *N. alata*—pp. 566, 573 (97, 98)
N. maritima × *N. attenuata*—pp. 569, 573 (60)
N. maritima × *N. debneyi*—p. 572 (98, 154)
N. maritima × *N. gossei*—p. 572 (98, 154)
N. maritima × *N. megalosiphon*—p. 572 (98)
N. maritima × *N. plumbaginifolia*—pp. 566, 573 (60)
N. maritima × *N. sanderae*—pp. 566, 573 (97, 98)
N. maritima × *N. velutina*—pp. 572 (97, 98, 154)
N. megalosiphon × *N. debneyi*—p. 572 (154)
N. megalosiphon × *N. gossei*—p. 572 (98, 154)
N. megalosiphon × *N. longiflora*—pp. 566, 573 (60)
N. megalosiphon × *N. plumbaginifolia*—pp. 566, 573 (60)
N. megalosiphon × *N. suaveolens*—p. 572 (97, 98, 154)
N. miersii × *N. bigelovii*—pp. 569 (97, 98)
N. miersii × *N. clevelandii*—p. 569 (98)
N. miersii × *N. langsdorffii*—pp. 566, 569 (97, 98)
N. miersii × *N. megalosiphon*—pp. 569, 573 (97, 98)
N. miersii × *N. suaveolens*—pp. 569, 573 (97, 98)
N. nesophila × *N. repanda*—p. 566 (60)
N. nesophila × *N. stocktonii*—p. 566 (60)
N. noctiflora × *N. alata*—pp. 566, 567 (97, 98)
N. noctiflora × *N. langsdorffii*—pp. 566, 567 (97, 98)
N. noctiflora × *N. petunioides*—p. 567 (60)
N. noctiflora × *N. sanderae*—pp. 566, 567 (97, 98)
N. nudicaulis × *N. glauca*—pp. 549, 569 (50, 60, 97, 98)
N. nudicaulis × *N. glutinosa*—pp. 555, 569 (17, 97, 98)
N. nudicaulis × *N. palmeri*—pp. 562, 569 (97, 98)
N. nudicaulis × *N. rustica*—pp. 551, 569 (97, 98)
N. nudicaulis × *N. suaveolens*—pp. 569, 573 (44, 97, 98)
N. nudicaulis × *N. tabacum*—pp. 556, 569 (44, 97, 98)
N. nudicaulis × *N. tomentosa*—pp. 555, 569 (97, 98)

N. tabacum × *N. sylvestris*—pp. 556, 557, 567 (20, 50, 70, 83, 97, 98, 132, 157)

N. tabacum × *N. tomentosa*—pp. 555, 556, 557 (20, 50, 70, 83, 97, 98)

N. tabacum × *N. tomentosiformis*—pp. 555, 556, 557 (5, 6, 10, 20, 50, 83, 97, 98)

N. thyrsiflora × *N. glauca*—pp. 549, 550 (60)

N. tomentosa × *N. setchellii*—p. 554 (60)

N. tomentosa × *N. tomentosiformis*—p. 554 (20, 60, 83, 97, 98)

N. tomentosiformis × *N. setchellii*—p. 554 (60)

N. trigonophylla × *N. nudicaulis*—pp. 562, 569 (17, 50, 97, 98)

N. trigonophylla × *N. palmeri*—p. 562 (97, 98)

N. trigonophylla × *N. setchellii*—pp. 555, 562 (60)

N. trigonophylla × *N. tomentosa*—pp. 555, 562 (50, 60, 97, 98)

N. trigonophylla × *N. tomentosiformis*—pp. 555, 562 (50, 60, 97, 98)

N. undulata × *N. arentsii*—p. 561 (57, 60)

N. undulata × *N. attenuata*—pp. 561, 569 (60)

N. undulata × *N. benavidesii*—p. 562 (60)

N. undulata × *N. cordifolia*—p. 562 (60)

N. undulata × *N. glutinosa*—pp. 555, 562 (60)

N. undulata × *N. langsdorffii*—p. 562, 566 (60)

N. undulata × *N. longiflora*—pp. 562, 566 (60)

N. undulata × *N. raimondii*—p. 562 (60)

N. undulata × *N. wigandioides*—p. 561 (57, 60)

N. velutina × *N. exigua*—p. 572 (97, 98, 154)

N. velutina × *N. gossei*—p. 572 (154)

N. velutina × *N. megalosiphon*—p. 572 (154)

N. velutina × *N. rotundifolia*—p. 572 (154)

N. velutina × *N. suaveolens*—p. 572 (97, 98, 154)

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TISSUE RESPONSES TO PHYSIOLOGICALLY ACTIVE SUBSTANCES

BETTY F. THOMSON

Connecticut College, New London, Conn.

INTRODUCTION

The normal course of development of most plant tissues and their contribution to the growth of the organism are well understood. In contrast to this is our lack of knowledge of the chemical factors which control the growth and differentiation of tissues. The identification of a number of naturally occurring and synthetically produced substances which exert a marked influence on growth has opened a new field of experimental plant histology. The object of this review is to summarize and evaluate the work thus far carried out in this field.

An early attempt to analyze the responses of plant tissues to specific chemical substances was made by Erwin Smith in 1917 (55). He performed experiments seeking to determine the mechanism of tumor growth resulting from infection with the crown-gall micro-organism, *Phytomonas tumefaciens*. The substances which he used were simple inorganic compounds, mostly ammonium salts. These were dissolved in water and injected into the hollow pith of *Ricinus* and similar stems. Cellular proliferations resulting from the compounds which he tested were chiefly callus-like, with some differentiation of vascular elements. After Smith's work there was little or no further activity in this line of investigation for a number of years.

Interest in plant hormone research developed in the late 1920's and from then on information on gross morphological responses of plants to hormones or hormone-like substances accumulated fairly rapidly. Boysen Jensen, Avery and Burkholder (12) discuss observations recorded up to 1936. By that date the list of observed structural responses to externally applied hormones included callus formation, tumor formation, adventitious rooting and cambial activation (also 58, 59, and later papers 15, 60, 61); fasciation is also reported (28). In 1936 the formation of large overgrowths resembling crown-galls was added to this list of hormone-induced phenomena (16). Kraus, Brown and Hamner (26) in the same

year presented the first of many detailed studies on the histology
of responses to local application of a number of synthetic growth
substances.

For the present review, 1936 is taken as a base-line; papers
appearing before that contain only scattered and incomplete data
on the histology of morphological changes induced by applying
growth substances. The extensive literature on bud inhibition,
formative effects, *etc.*, is outside the intended scope of this paper.

The terms "hormone", "auxin" and "growth substance" are used
interchangeably; no subtle distinctions are intended. Throughout
this paper the names of the most frequently used compounds are
abbreviated as follows: indoleacetic acid, IAA; indolebutyric acid,
IBA; indolepropionic acid, IPA; naphthaleneacetic acid, NAA;
naphthoxyacetic acid, NOA; phenylacetic acid, PAA; and naphtha-
lene acetamide, NAD.

EFFECT OF GROWTH SUBSTANCES APPLIED TO VARIOUS PARTS
OF THE PLANT

Young decapitated stems

Responses of different species to IAA. Of known growth sub-
stances, IAA has been tested on the greatest number of species.
Many studies have been made on plants decapitated in the internode
above the first foliage leaves when that internode was still elon-
gating; the growth substance mixed with lanolin (mostly 0.5% to
2.0%) was then applied to the cut stump.

The responses of kidney bean (26), broad bean (48), pea (52),
tomato (11), cabbage (21) and sunflower (10) to such treatment
with IAA differ from one another chiefly in detail. Generally the
tissues most reactive to IAA are endodermis, phloem, rays and pith.
Cambial divisions may be stimulated and maturation of the resulting
cells delayed. Cortex and xylem parenchyma may undergo much
or little proliferation. Epidermis and pericycle usually show little
or no response.

In the tissue masses resulting from proliferation induced by IAA,
some regions may remain meristematic indefinitely; some organize
as root primordia; some differentiate as more or less organized
patches or strands of vascular elements which may remain isolated
or anastomose and eventually connect with the main stele of the
original stem.

Hormone-induced root primordia may arise from derivatives of cortex, endodermis, phloem, rays, or pith, or combinations of these. Commonly, the root caps of such roots consist of endodermal derivatives.

On the cut surface of decapitated stems to which the hormone-lanolin mixture has been applied, a mass of callus tissue may appear. The extent, form and origin of this callus varies with the species as well as with the hormone used.

In IAA-treated decapitated stems of etiolated peas (52), all tissues from inner cortex to xylem parenchyma proliferate; this results in the formation of a meristematic cylinder from which are differentiated endodermis with casparian strips, pitted tracheids and much parenchyma. *Iresine* (24) and *Mirabilis* (22) differ from most species studied in that the pericycle is more reactive than the endodermis; adventitious roots arise mostly from pericycle and ray tissues. The phloem of *Iresine* is strongly reactive, that of *Mirabilis* is not. Neither of these species produces much callus.

Some plants produce unique responses. For example, in *Vicia faba* (48) roots are rarely formed, but queer "proliferations" occur; these are unorganized masses of small but vacuolated cells, which look as though root primordia start to develop but do not become completely organized. Cabbage (20, 21) is unusual in producing adventitious shoots, apical ones from callus, lateral ones from inner cortex and rays; primordia of these lateral shoots can be distinguished from root primordia in early stages only by their lack of root caps.

Among three species of lily (5), differences in response occur for which there is no visible anatomical basis. In *L. longiflorum* and *L. philippinense formosanum* patches of parenchyma adjacent to the phloem of vascular bundles become meristematic, and the new tissue masses formed differentiate into root primordia. In the anatomically similar and taxonomically closely related *L. Harrisii* no such change occurs, but rather the epidermis and adjacent tissues in the leaf axils become meristematic and give rise to bulbils.

Summary for IAA on decapitated stems of different species. The response of decapitated stems of different species of plants to relatively high concentrations (ca. 1–2%) of IAA in lanolin consists basically of cellular proliferation. Endodermis, phloem, rays

and pith are generally more reactive than other tissues. Vascular strands may differentiate within these proliferated tissues, or the proliferated tissues may organize into root primordia. In rare cases the newly formed tissues differentiate into adventitious shoots, as in cabbage and one species of lily. Superficial cell divisions may lead to callus formation.

Responses of a single species to different growth substances. The reactions of a single species to different growth substances are varied. A number of substances have been tried on the red kidney bean, *Phaseolus vulgaris.* IAA (26) brings about abundant proliferation in endodermis, phloem and pith, and formation of an irregular mass of callus on the cut surface. IBA (23) produces similar but less marked results; adventitious roots are characteristically broad and heavy. NAA (23) produces a smaller callus, broad and flat-topped, and root primordia appear as a ring of large bumps at the base of one-cm.-long ridges (formed by endodermal proliferation over the vascular bundles). IPA produces witches' brooms, a type of fasciation (16).

Bean stems treated with tryptophan (25) form no roots. Specific tissue responses are less vigorous but essentially similar to those described above. Noteworthy is the formation of abundant anastomosing vascular strands which differentiate mostly from endodermal derivatives. NAD (27) brings about relatively little proliferation of any tissues; production of secondary xylem is increased, and the walls of all kinds of cells become much thickened. Tetrahydrofurfuryl butyrate (45) has little or no effect on epidermis, cortex, pericycle or cambium, and causes the endodermis to produce only a narrow band of parenchyma with no vascular differentiation; xylem, phloem and rays proliferate, producing a callus ring which flares out and down around the top of the stem. An extract of maize pollen (44) applied to bean stems produces results similar to those from tryptophan, the chief response being proliferation of cortical parenchyma and endodermis.

Sunflower has been treated with several growth substances (10). IBA induces proliferation chiefly in endodermis and pith. IPA causes bulging of the stem due to proliferation of cortex and pith. PAA and NAA stimulate especially the cambium; NAA also induces the formation of a unique horizontal cambium near and parallel to the cut surface.

Summary for different substances on decapitated stems of a single species. It is not possible to predict the kind of response that a given species will make to different growth substances; the only consistent response is cellular proliferation, and this varies as to the tissues in which it is induced, relative intensity, *etc.*

Young intact stems

Growth substances applied in lanolin to elongating stems of intact plants bring about reactions essentially similar to those of decapitated plants. In the case of beans treated with IAA (23), the response is the same as that of decapitated plants, except that there is no reaction in the pith. A lanolin mixture of maize pollen extract (44) applied to intact bean stems produces an effect like that of 0.002% IAA: elongation of cells in the pith, tapering from a maximum of 246% longer than controls at the point of application to no elongation above and below the site of treatment. A lanolin suspension of extract of crown-gall bacteria produces essentially the same reaction on bean stems as 3% IAA (32). NOA applied in lanolin to tomato stems produces results similar to those of IAA (4): epidermis and cortical parenchyma proliferate moderately; the most responsive tissue is the endodermis; a cylinder extending from endodermis to cambium becomes meristematic and gives rise to root primordia in which the root tips form from derivatives of pericycle and phloem, the root caps from those of the endodermis.

Meristems

The response of meristematic regions to the application of growth substances differs considerably from that of older tissues. Growth substances (0.3–1.0% of IAA, IPA, IBA, NAA) applied in lanolin delay the development of the stem apex and of very young leaf primordia of *Tradescantia* (9); treatment of the internode at the region of rapid elongation causes differentiation of many fasciated roots and vascular elements in the cortical region; treating nearly mature regions stimulates the growth of existing root primordia and the production of many new ones.

Treatment of tomato stems near the apex with NOA in lanolin (4) inhibits cell division in the meristem; a 1% mixture causes injury and necrosis, 0.1% inhibits growth and causes differentiation to the very tip of the apex in six days. The stronger mixture

inhibits growth when applied to the region of rapid elongation or to the region where secondary growth is well established; these regions are stimulated to proliferate by the more dilute mixture.

Minute droplets of hormone-lanolin mixture (0.1, 1.0, 3.0, 6.0% of IAA or IPA), applied directly to the stem apex of *Tropaeolum* (2), inhibit development of neither terminal nor axillary buds; leaf primordia and the leaves developing from them become fasciated and much enlarged tangentially, and phyllotaxis is disturbed; no tumors or adventitious roots are produced. When IAA (0.05 or 0.077% in lanolin) is applied to stem apices of *Lupinus* or *Epilobium*, the next primordia to develop are often united, or primordia arising from points near the site of application may be displaced toward the treated region (57); the primordia from treated spots and their axillary buds are frequently abnormally large.

Summary for shoot meristems. Treatment of meristems with growth substances either retards or inhibits their growth, or else upsets their normal behavior, often causing enlargement and displacement of primordia.

Roots

The roots of bean seedlings treated with as little as 0.5 ppm of IAA in lanolin bend toward the point of application, indicating a local inhibition of growth at the site of treatment (32); higher concentrations cause sharper bending, sometimes to the extent of coiling, and retard root elongation; 0.01% to 3% mixtures may completely stop elongation and cause local swellings at the root tip which may rupture the epidermis. Lateral roots form prematurely in all these cases. These responses are obtained only by treating within 3 mm. of the root tip.

Application of growth substances (IAA, IPA, IBA, NAA, at 0.01% to 1% in lanolin) to the tip or to a point 1.5 or 3 cm. behind the tip of intact aerial roots of the tropical vine *Cissus* (49) retards elongation; this is accompanied by an increase in diameter due to enlargement of cells (also nuclei) in phloem, cortex and pith. Lateral roots arise from the pericycle, as they do when the untreated root strikes into a solid substratum in its natural habitat.

Excised tomato roots growing *in vitro* (18) produce no tumors in response to indole (0.1–200 mg./1), tryptophan (10^{-4} to 10^{-9} molar), acetic acid (10^{-2} to 10^{-8} molar) or variations in pH. Ether

extracts of the bacterium *Phytomonas tumefaciens* cause slight tumor formation. IAA produces definite swellings or galls when applied in concentrations which inhibit elongation (10.0–0.0125 mg. per liter). Such galls show hypertrophy of epidermis and cortex, production of many root primordia from the pericycle, excessive development of root hairs, and callus formation on the cut ends of the roots. Normal growth is resumed when the roots are transferred to fresh nutrient medium.

Roots of monocots (onion, narcissus, tulip) immersed in 10 to 20 ppm of growth substance (NAA, IAA, IBA, NOA, NAD, tryptophan) in nutrient solution cease elongating (17). Onion produces secondary roots in excess. Onion and narcissus form tumors near the root tip by enlargement of cortical cells in response to NAA, IAA, IBA, NOA and NAD; no tumors result from treatment with tryptophan. In another study (29) it was observed that elongation of onion roots is inhibited by placing them in solutions of 1 to 10 ppm of NAA or IBA. IBA causes much and NAA a little swelling of cortical cells, resulting in the formation of a tumor near the root tip. Often cells of the apical meristem cease dividing; in this case lateral root primordia arise from the pericycle.

Summary for roots. Growth substances applied to roots in concentrations high enough to inhibit elongation cause marked swelling of relatively embryonic cells near the root tip; this is accompanied by production of lateral roots from the pericycle.

Effect at a distance from site of application ("telemorphic" effect)

Application of 1% 4-chlorophenoxyacetic acid in lanolin to intact leaflets or internodes of sweet pea seedlings produces a "telemorphic" effect in the roots of the treated plants (8). The internode above the site of treatment becomes pale and thickened, and the roots swell for a variable distance just behind the root tip due to extensive proliferation in the pericycle and some in the endodermis. This substance is the only one of several tried which produced such an effect-at-a-distance.

Fruits

Fruit tissues show a wide range of response to treatment with growth hormones. The structure of holly fruits which develop

parthenocarpically after spraying the female flowers with 0.04% aqueous IAA closely resembles that of fruits resulting from pollination (19); the only discernible difference is a slight proliferation of treated stigma cells and a delay in their collapse and suberization.

When the stigma and style are removed from pistils of *Lilium regale* by a clean cut at the top of the ovary and the exposed surface is coated with a hormone-lanolin mixture (1% IAA, NAA, NOA), the fruits undergo partial parthenocarpic development (7). The ovaries continue to enlarge for some time due to swelling of cells in the outer carpel walls and outer integuments of the ovules. This growth ceases after about three weeks, and soon afterward the fruits become pale and pithy, due to death and dissolution of the inner carpel walls. The whole fruit dies after eight to ten weeks, being then smaller than pollinated fruits of the same age. Cucumbers decapitated similarly at the base of the style and smeared with 2% IAA in lanolin behave otherwise (66). When treated at full bloom, the apical tissues remain alive but make scant proliferation; treated about four days before full bloom, tissues of nectary, floral tube and neck proliferate to form a small apical tumor in which no vascular tissues nor root primordia appear.

Bean pods developing after pollination respond to IAA in lanolin by callus formation and rooting from the callus, as in the case of decapitated stems of bean (23). Underlying tissues were exposed by cutting off the terminal quarter of the pod or by abrading the suture edge with emery cloth; in either case the raw surface was coated with the lanolin mixture.

Summary for fruits. Growth substances can apparently substitute with varying degrees of completeness for pollination as a stimulus for fruit development. If the fruit has begun growth following pollination, its tissues respond to hormone treatment like other green tissues of the plant, *i.e.*, produce callus and roots, at least in the case of the kidney bean.

DELAY OF MATURATION OF TISSUES BY APPLICATION OF GROWTH SUBSTANCES

De-blading of *Coleus* leaves accelerates cell division in the absciss layer, resulting in the early shedding of the remaining petiole. Treatment of the cut stump of the petiole with lanolin containing IAA, IPA or IBA retards development of the absciss layer and

consequently delays abscission (46). Similarly, addition of 0.2–10 mg. per liter of IAA or NAA to tobacco tissue cultures inhibits differentiation of the tissue (53). (This effect can be offset by changes in the composition of the culture medium.) Maturation of cambial derivatives is sometimes delayed after treatment with growth substances, for example, in bean stems treated with IAA (26) or NAA (23) and cabbage treated with IAA (21).

FACTORS AFFECTING THE RESPONSES OF TISSUES

Deficiency of carbohydrate prevents or at least retards the tissue responses here discussed (3, 6, 56). This is understandable, since plants cannot build without building materials, even in response to such a profound stimulus as treatment with a physiologically active substance.

Nitrogen-deficient cabbage plants show similar but slower reactions to treatment with growth hormones than do those given a complete nutrient (21). Bean plants on a low nitrogen, high carbon "diet" show faster rooting than plants maintained on the reverse ratio (56). Nitrogen-deficient tomatoes respond as well to NOA as do those grown with an adequate nitrogen supply (3). Apparently, carbohydrate deficiency is a more severely limiting factor than lack of nitrogen in this type of response.

Stems of etiolated bean plants show only slight elongation but marked thickening when treated with 3% IAA in lanolin (40), while controls continue their spindly habit. In etiolated bean plants treated with a mixture of one part ether extract of maize pollen in four parts of lanolin (44) there is little or no difference between experimental and control plants with respect to internode elongation; in light-grown plants the pollen extract produces greater elongation than pure lanolin. The latter response resembles the reaction of plants to very dilute (0.002%) IAA.

The age of the treated tissue affects the nature of the response. Treated meristems continue to produce new cells, provided the concentration of applied hormone is not great enough to cause actual injury, although the developmental pattern may be altered (2, 57). In older tissues, only those cells respond which are sufficiently undifferentiated to be able to revert to the embryonic state, such as cambium and parenchyma-type cells. The proportion of such cells decreases as the tissues and organs mature, and

experiments show that older regions of a plant respond less vigorously to auxin application than do younger ones (21, 22, 24).

RELATION OF GROWTH SUBSTANCES TO CROWN-GALL

It was established by Erwin Smith in 1911 (54) that the peculiar masses of histologically chaotic tissue known as crown-galls are due to infection with a species of bacterium, *Phytomonas tumefaciens*. The idea that crown-galls result from a specific chemical substance formed by the infecting organism also goes back to Smith in a paper of 1917 (55). A relation between growth hormones and crown-gall became apparent in 1936 when Brown and Gardner (16) reported the formation of large tumors resembling crown-galls on several species of plants as a result of smearing punctured areas on the stems with a 2% mixture of IAA in lanolin; similar galls were obtained by treating with a lanolin mixture of an ether extract of *P. tumefaciens* cells grown in pure culture. From that point, investigations proceeded in two directions: one concerning the responses of plant tissues to synthetic growth substances, discussed in other sections of this paper; the other concerning the relation of hormones to the crown-gall disease.

Histological examination of tumors formed on bean plants in response to treatment with 3% IAA, or to an ether extract of *P. tumefaciens,* or to inoculation with living cells of this organism shows that tumors from all three of these causes have a similar structure (31, 32). (See above for details of tissue responses.) This fact and the observation that other plant reactions which follow auxin treatment, *e.g.,* epinasty, adventitious rooting, cambial stimulation, *etc.* (33, 34), are associated with growth of crown-galls, indicate further that crown-gall formation is correlated with an increase in the auxin content of the infected tissues.

Investigations of the actual auxin content of galls, as compared to normal tissue, yield conflicting data. Using the *Avena* test, Locke, Riker and Duggar (34) could detect no auxin in the tissue of old galls, using either the diffusion method or extraction with chloroform and alcohol; diffusion tests made on a few tomato seedlings indicated that more auxin is present in crown-gall-inoculated than in control tissues. Later Riker (50) and Riker, Henry and Duggar (51) reported that the amount of auxin extracted by ether from galls (50) or from crown-gall-inoculated tissues (51) is only

slightly greater than that obtained from control tissues, and that this small difference disappears when the yields are expressed in relation to total nitrogen per gram of tissue. No difference in auxin content was found between gall-inoculated plants kept at 27° C, where galls develop, and those kept at 31°, where they do not (51). Link and Eggers (30), on the other hand, found that by various methods of ether extraction, more auxin, both free and potential, is obtained from gall-inoculated than from healthy tissues. It may be that the development of a fully reliable method of total auxin extraction will resolve these differences. Link and Eggers state (30) that none of the methods tried in their tests and others is satisfactory for the determination of total auxins of tomato.

Whatever the relative hormone content of tumor tissue, an adequate auxin supply seems to be necessary for the full development of crown-galls. Tissues inoculated with an attenuated strain of *P. tumefaciens* usually fail to proliferate, at least to any extent. However, if such tissues are supplied with additional growth hormone, typical large galls result (14, 33, 34, 50). The extra hormone may be effectively supplied from various sources: as growth substance diffusing either from expanding axillary foliage above the site of inoculation or from gall tissue formed higher up on the stem in response to inoculation with a virulent strain, or as a synthetic hormone applied in lanolin to a decapitated stem. IAA, IBA and NAA are active in this case, IAA less so than the others. Bacteria isolated from full-sized galls produced in this way are found to be still attenuated. It has been suggested (14) that gall production takes place in two phases: first, normal cells of the host plant are changed into tumor cells (but as yet do not proliferate) ; then growth substances stimulate the genetically altered tumor cells to divide. Virulent crown-gall bacteria bring about both of these phases, attenuated strains only the first phase.

The origin of the growth substances involved in the production of crown-galls by virulent bacteria has not been clearly determined. The identification of IAA or a closely related compound in an extract of crown-gall bacteria (31) at first suggested that *P. tumefaciens* incites tissue proliferation by virtue of the IAA it presumably secretes into the host plant. However, experiments show that there is no significant difference in the amount of growth substance produced in peptone broth by virulent and by attenuated crown-

gall bacteria and by the non-pathogenic *Bacillus radiobacter* (34, 35); the auxin in these three cases is of the same type as IAA in its reaction to hot acids and alkalis (35). It was also found (36, 50) that auxins obtained from bacterial cells, from tomato galls and from normal tomato tissues are all of the same type, again like IAA in reaction to acid and alkali. This makes it at present impossible to determine experimentally whether the auxin comes from the host or from the parasite. However, the concentration of IAA formed by even a large mass of bacteria in culture is far less (ca. 125 micrograms per liter) than that required to cause proliferation when applied in lanolin (*e.g.,* 1% equals 10,000,000 micrograms per liter) (51). In view of the known facts, it seems highly probable that the auxin involved in crown-gall proliferation is formed by the host cells in response to a bacterial stimulus, the nature of which is at present unknown.

The change from normal to tumor cells is accomplished before any visible proliferation occurs. When periwinkle plants are inoculated with a fully virulent culture of crown-gall bacteria and the bacteria killed by heat treatment (placing the plants in a room held at 46°–47° C) 24 to 36 hours after inoculation (13), small galls are subsequently produced which resemble those resulting from an attenuated strain of bacteria. It would be interesting to know whether these would grow into large galls if additional hormones were supplied. When normal cells have once been transformed into tumor cells by exposure to the bacterial stimulus, they remain so, even after the stimulus is removed, *i.e.,* the galls are self-perpetuating. This is true whether the initiating bacteria are of a virulent or an attenuated strain. The permanence of the change in cells is abundantly demonstrated by the work of White and Braun on tissue cultures and transplants of tissue from bacteria-free secondary galls (14, 64, 65).

Summary for auxins and crown-galls. The histological likeness of auxin-induced tumors to crown-galls and the fact that plant reactions which follow application of auxins also accompany crown-gall formation indicate that crown-gall development is associated with increased auxin content. The actual total auxin content of galls as compared to normal tissue has not yet been clearly established. Attenuated crown-gall bacteria produce large galls when extra growth substance is supplied to the infected tissue. The

auxin involved in normal gall development appears to come from the host tissue in response to an unknown bacterial stimulus.

MECHANISM OF RESPONSE

The precise manner of auxin action in the tissue responses here discussed is not clear. The available experimental data seem most satisfactorily interpreted by assuming that the basic role of the applied hormone is to bring about a mobilization of materials toward the site of treatment. There are some quantitative experimental data supporting this viewpoint. For example, when the cut bases of bean seedlings are placed in vessels of aqueous auxin solution, the dry weight of the hypocotyls increases over that of controls in water (42, 62). When auxin is applied in lanolin locally, there is an increase in the fresh and dry weights of the tissues in the treated region (38, 40, 41). Carbohydrate specifically is transported to the region of treatment (1). It has also been shown that protein accumulates in regions which become meristematic in response to local auxin application and that this increase precedes the beginning of cell division (11). The accumulation of materials and consequently the growth of parts of plants above the regions treated with growth substances is sharply restricted (37, 40, 62)—a further indication of the control that auxins exert over the direction of transport.

The effect of auxin applied to stem apices of some species (2, 28, 57) can be explained on the basis of an attraction of additional nutrients to the meristem, causing derangements in growth leading to over-large primordia and leaves, and sometimes to fasciation. Bacteria-caused crown-galls likewise involve an accumulation of solid matter at the site of infection.

The disposition of the mobilized material varies with the species, the substance applied and the site and mode of application. The mobilized material may be added to cell walls in the transformation of pith or ray cells to tracheids (26, 52) or in a general thickening of all cell walls in addition to increased secondary growth, as in response to NAD (27); or it may be added to cytoplasm and nucleus; this is often followed by cell division. A new mass of cells thus produced may take various forms: the cells may remain meristematic and continue to divide indefinitely; or they may differentiate in any of several ways: as relatively undifferentiated callus

tissue, as vascular tissue in patches or strands, as adventitious root primordia which may grow into functional roots or not, or rarely as adventitious shoots (cabbage (20, 21) and one species of lily (5)).

Various experiments have been carried out in an effort to determine more precisely the mechanism of the action of auxins in bringing about the tissue reactions under discussion. There is ample evidence that the rate of starch hydrolysis is increased in treated regions (39, 43, 62, 63). This would make carbohydrates more readily available for growth processes. Protoplasmic viscosity is reduced in bean stems after application of IAA, IPA or NAA, presumably because of dissociation of cellular proteins (47); this is believed by the investigator to cause an increase of protoplasmic swelling pressure, of respiration and of polysaccharide hydrolysis. Results of investigations on respiration rates in plants undergoing tissue changes in response to auxin treatment are not entirely consistent. In one study (40) no difference was found between respiration of intact control plants and that of intact plants treated with 3% IAA in lanolin, whether measured by loss of dry weight or by the amount of carbon dioxide respired. Another investigator (1) found that the same mixture applied to decapitated plants caused a decrease in dry weight and in starch content of the entire plant as compared to controls. Intact bean plants treated with NAA or NAD in lanolin contain less starch, dextrin and sugars than controls (37), also indicating an increase in respiration. Other investigations of the effect of auxins on respiration have been made on different plant materials and are not strictly pertinent here.

CONCLUSION

Summaries of experimental details appear at the ends of the various sections of this review and need not be repeated here.

The most consistent response of plant tissues to treatment with relatively high concentrations of physiologically active substances is cellular proliferation. The specific tissues which respond and the nature and degree of the response vary with both the species and the substance applied. Treatment of tissues which are already proliferating, such as meristems, usually causes either inhibition of growth or a distortion of the normal growth pattern.

Crown-gall infection causes abnormal growth resembling that

induced by synthetic auxins.   The precise relation of the presence of bacteria to the auxins which appear to be involved in gall development remains to be determined.

The action of auxins in the tissue responses here discussed has been interpreted as a mobilization of solid matter toward the site of treatment.   The fundamental biochemistry of auxin action in this and other phenomena is not as yet understood.

The exploratory work discussed above has opened an important field of inquiry; but it does not seem that further accumulation of similar data will contribute to a basic understanding of these phenomena and their significance to growth and development.   A fresh approach is clearly needed.

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